FORMULATION AND CHARACTERIZATION OF FLOATING MICROSPHERES OF PERINDOPRIL ERBUMINE

Dissertation submitted to

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CERTIFICATE

This is to certify that the dissertation entitled **"FORMULATION AND CHARACTERIZATION OF FLOATING MICROSPHERES OF PERINDOPRIL ERBUMINE"** submitted by **Mr. S. KATHIRAVAN** in partial fulfillment of the requirement for the Degree of **Master of Pharmacy** in **Pharmaceutics** is a bonafide work carried out by him, under the guidance and supervision of **Mr.A.Abdul Hasan Sathali, M.Pharm.,(Ph.D)** Professor and Head, in the Department of Pharmaceutics, Madurai Medical College, Madurai-20, during the academic year 2011 – 2012. This dissertation is forwarded to the Controller of Examination, The Tamil nadu Dr. M.G.R. Medical University, Chennai.

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I wish him success in all his endeavors.

Place: Madurai **(Prof. Mr. A. Abdul Hasan Sathali)**

Date:

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CONTENTS

CHAPTER-I

INTRODUCTION

ORAL CONTROLLED DRUG DELIVERY SYSTEMS

Oral ingestion has long been the most convenient and commonly employed route of drug delivery. Indeed, for controlled release systems the oral route of administration has received attention with respect to research on physiological and drug constraints as well as design and testing of product. This is because there is more flexibility in dosage form design for oral route than that of parenteral route. Drug delivery technologies are the formulation technologies that modify drug release profile, absorption, distribution and elimination for the benefit of improving product efficacy and safety, as well as patient convenience and compliance (Brahmankar.D.M., Jaiswal.S.B., 2007).

Routes of administration

1. Enteral.
2. Topical.
3. Parenteral.

1. Enteral drug delivery:

It includes peroral i.e.

- ✓ Gastro-intestinal
- ✓ Sub-lingual
- ✓ Rectal

2. Topical drug delivery:

It includes skin, eyes or other membranes.

- ✓ Intranasal
- ✓ Inhalational
- ✓ Intravaginal
- ✓ Transdermal

3. Parenteral drug delivery:

It includes all routes of administration through or under one or more layers of skin.

- ✓ Intramuscular
- ✓ Subcutaneous
- ✓ Intravenous

The most preferred route of drug administration for systemic delivery of drugs is orally (Chien, Y.W., 1982). More than 50% of drug delivery systems available in the market are oral drug delivery systems. These systems have the obvious advantages of case of administration and patient acceptance. Several oral drug delivery technologies have come and gone, and new systems still emerge even today.

One would always like to have ideal drug delivery systems that will possess two main properties (**Lingaraj, *et al*, 2010),**

1. It will be a single dose for the whole duration of treatment,
2. It will deliver the active drug directly at the site of action.

The Challenge:

Most of the marketed products currently available are immediate release products. To achieve and maintain the concentration of an administered drug within therapeutically effective range (Vyas.S.P.,Khar R K., 2002. Chien, Y.W., 1982.

Brahmankar.D.M., Jaiswal.S.B., 2007) it is often necessary to take drug dosage several times and this result in a fluctuating drug level in plasma**.**

The Controlled Release:

- Controlled drug delivery is one which delivers the drug at a predetermined rate, for locally or systemically, for a specified period of time.
- Continuous oral delivery of drugs at predictable & reproducible kinetics for predetermined period throughout the course of GIT.

There are many benefits offered by controlled drug delivery systems **(**Chien, Y.W., 1982**)**. For example, sustained release technologies allow prolonged delivery of a therapeutic dose, thus reducing the number of times that a patient needs to take their medication while maintaining a steady state of drug in the bloodstream, and time-delayed release introduces a lag time before dose release, providing pulsatile delivery of drug to specific sites, such as the colon, or at a specific time. Temporal control of drug release has particular advantages in the treatment of disorders that demonstrate a circadian pattern, such as cardiovascular disorders, asthma, anxiety and hypercholesterolemia. In such cases, the development of controlled-release formulations that deliver the payload at an optimal time can greatly enhance the therapeutic effects of the drug and reduce the dose required.

Potential methods that can be used to retard drug release are:

1. Capsules of polymeric material filled with a solid or liquid drug or with a suspension of drug, in which drug release is controlled by diffusion through the capsule wall.

2. A heterogeneous dispersion of drug particles in a solid matrix which can be either biodegradable or non-biodegradable, and drug release is controlled by diffusion through or erosion of the matrix, or both.

3. A laminate of agent and polymeric material made by coating a film of biodegradable material with solid drug and then by forming the filminto a sealed "Sandwich" or "Jelly roll" in which drug release is controlled by diffusion, erosion, or both**(**Robinson J.R. and Lee V.H.L., 1987.).

4. Liquid-liquid encapsulation of the drug in a viscous solution of polymer, in which drug release is controlled by slow diffusion through dilution of the media **(Zhao W.Q., Pu B.Y. and Hartland S., 1993)**.

5. Pumps that either mechanically or chemically (osmotic pressure) provide drug in a controlled manner (**Prabakaran D., *et al*., 2004, Li X., Pan W., Nie S. and Wu L., 2004. Verma R.K. and Garg S., 2004**).

6. Microparticles that have an apparent density lower than that of gastric juice. Thus, the final product float on gastric juice for an extended period while slowly releasing the drug **(Streubel A., *et al*., 2002, EL-Kamel A.H., 2001).**

7. Drug-containing bio adhesive polymer that adheres to the mucin coating of the gastrointestinal tract (GIT) and retained on the surface epithelium to extend GI

transit time of the drug. Drug is released at a constant rate from the bio adhesive polymer for subsequent absorption **(Kriwet B *et al*., 1998)**

8. Chemical bonding of drug to a polymer backbone by pendent amide or ester linkage, in which drug release is controlled by hydrolysis (**Hoste K., *et al* 2004**)

ORAL CONTROLLED RELEASE FORMULATIONS:

Oral route has been the commonly selected and most convenient for the drug delivery. **(**Vyas.S.P., Khar R K., 2002**)** Oral route of administration has more attention in the pharmaceutical field because of the more flexibility in the designing of dosage form than routes drug delivery.

Most of the oral controlled drug delivery systems rely on diffusion, dissolution or combination of both mechanisms, to release the drug in a controlled manner to the Gastrointestinal Tract (GIT).

Novel oral drug delivery systems are broadly classified in to two categories as they may controlled release dosage forms as well as targeting dosage forms. General controlled manner in the GIT for systemic uptake and no particular area of GIT specified. In contrast, targeted preparations are releasing the drug in a specified area or tissue of the GIT (e.g. colon specific drug delivery systems).

Targeting systems are either releasing drug in controlled manner or in one burst at the specific area (Vyas, S.P., Khar, and R.K). The goal of a targeted oral drug delivery system (TODDS) is to achieve better therapeutics success compared to conventional dosage form. This can be achieved by improving the pharmacokinetic profile, patient convenience and compliance in therapy, some of the advantages of TODDS are:

- ✓ Reduced dosing frequency
- ✓ Better patient convenience and compliance
- ✓ Reduced GI side effects and other toxic effects.
- ✓ Less fluctuating plasma drug level
- ✓ More uniform drug effect
- ✓ Less total dose
- ✓ Better stability of the drug.

On the other hand TODDS suffer from a number of potential disadvantages:

- o Higher cost
- o Relatively poor in vitro-in vivo correlation
- o Possible dose dumping
- o Reduced potential for dose change or withdrawal in the event of toxicity

Targeting of drugs through oral route involves control of time of release or location of release. On the basis of environmental, anatomical and physiological factors these drug delivery system can be classified with respect to target site as follows:

- ➢ **Systems targeted to stomach/duodenum**
- ➢ Systems targeted to small intestine
- ➢ Systems targeted to large intestine/colon
- ➢ Systems targeted to lymphatic.

Oral Diffusion-Controlled Systems:

The basic concepts of oral controlled release dosage forms can be defined based on release-profile characteristic or the underlying release- controlling mechanism (Xiaoling Li, Bhaskara R. Jasti., 2005). Two distinct drug release profiles, extended and delayed release, are achievable, and they can be used in various combinations to

provide the desired release rate. Three delivery systems dominate today's market of oral CR products:

- Matrix systems.
- reservoir systems and
- osmotic systems.

Release mechanisms from these dosage forms, diffusion plays a key role in both matrix and reservoir systems, whereas osmotic pressure is the predominant mechanism of drug release from osmotic systems and could also play a role in a reservoir system.

Matrix systems

A matrix system consists of active and inactive ingredients that are homogeneously mixed in the dosage form. Matrix systems divide into two categories, based on rate-controlling materials.

- ✓ **Hydrophobic matrix systems**
- ✓ **Hydrophilic matrix systems**

Hydrophobic matrix systems:

This is the only system where use of a polymer is not essential to provide controlled drug release, although insoluble polymers have been used. As the term suggests, the primary rate-controlling components of a hydrophobic matrix are water insoluble in nature. These ingredients include waxes, glycerides, fatty acids, and polymeric materials such as ethyl cellulose and methacrylate copolymers. To modulate drug release, it is necessary to incorporate soluble ingredients such as lactose into the formulation.

The presence of insoluble ingredients in the formulations helps to maintain the physical dimension of a hydrophobic matrix during drug release. Diffusion of the active form from the system is the release mechanism. Very often, pores form within a hydrophobic matrix as a result of the release of the active ingredient. Hydrophobic matrix systems generally are not suitable for insoluble drugs because the concentration gradient is too low to render drug release.

Hydrophilic matrix systems

The primary rate-controlling ingredients of a hydrophilic matrix are polymers that would swell on contact with the aqueous solution and form a gel layer on the surface of the system.

Drugs release from hydrophilic matrices is by polymer dissolution (erosion) and diffusion of drug molecules across the gel layer have been identified as the rate-controlling mechanisms.

The model semi empirical "exponent equation" has been used widely to differentiate the contributions of both mechanisms:

$$Q_t = kt^n$$

Where Q_t is amount Q in time t, *n* is a diffusion exponent, and *k* is a kinetic constant. If diffusion dominates polymer erosion, the value of *n* would approach 0.5. On the other hand, for erosion-controlled formulations, *n* would approach the value of unity. Under an "anomalous" condition, the value of *n* falls in between 0.5 and 1 when both diffusion and erosion play roles.

For very soluble compounds, diffusion of drug molecules is the dominant mechanism of release, and the role of polymer erosion is limited in modulating drug release. Thus, developing a hydrophilic matrix for highly soluble drugs that requires

prolonged release (e.g., >12 h) can be challenging. On the other hand, release of less soluble drugs from hydrophilic matrices is expected to be slow because both polymer dissolution and drug diffusion play key roles (Xiaoling Li, Bhaskara R. Jasti., 2005):.

Classification of oral controlled drug delivery system

1. **Continuous release system**
    1. Dissolution controlled release system
    2. Diffusion controlled release system
    3. Diffusion and dissolution controlled release system.
    4. ion exchange resin drug complexes
    5. slow dissolving salt and complexes
    6. pH independent formulations.
    7. Osmotic pressure controlled systems
    8. Hydrodynamic pressure controlled systems.

2. **Delayed transit and continuous release systems**
    1. Altered density system.
    2. Mucoadhesive system.
    3. Size based systems.

3. **Delayed Release system**
    1. Intestinal release system.
    2. Colonic release system.

Factors influencing the design and performance of controlled drug delivery system (Vyas.S.P.,Khar R K, 2002. Vyas, S.P., Khar, R.K., Xiaoling Li, Bhaskara R. Jasti., 2005).

a) **Biopharmaceutic characteristic of the drug**

1. Molecular weight of the drug
2. Aqueous solubility of the drug
3. Apparent partition coefficient
4. Drug Pka and ionization physiological pH
5. Drug stability
6. Mechanism and site of absorption
7. Route of administration.

b) **Pharmacokinetic characteristic of the drug**

1. Absorption rate
2. Elimination half life
3. Rate of metabolism
4. Dosage form index

c) **Pharmacodynamic characteristic of the drug**

1. Therapeutic range
2. Therapeutic index
3. Plasma–concentration–response relationship

Advantages of controlled drug delivery systems:

- Improved patient convenience and compliance
- Reduction in fluctuation in steady state levels.

- Increased safety margin of high potency drugs.
- Reduction in dose.
- Reduction in health care cost.
- Total dose is low.
- Reduced GI side effects.
- Reduced dosing frequency.
- Better patient acceptance and compliance.
- Less fluctuation at plasma drug levels.
- More uniform drug effect
- Improved efficacy/safety ratio.
- Dose dumping.
- Reduced potential for accurate dose adjustment.
- Need of additional patient education.

Disadvantages of controlled drug delivery systems

- Decreased systemic availability.
- Poor *invitro-invivo* correlations.
- Chances of dose dumping.
- Dose withdrawal is not possible.
- Higher cost of formulation.

Microencapsulation Techniques

Coacervation:

It is the earliest process used to make an encapsulated product. This process utilized the interaction of two oppositely charged polyelectrolyte in water to form a polymer–rich coating solution called a coacervate (Leon Lachman, Lieberman H A, Kanig J L). Both water soluble and insoluble drugs can be microencapsulated.

***Interfacial Polymerization*:**

It involves dispersing the organic phase containing drug particles into the aqueous phase containing monomers, whereby monomers react at liquid / liquid interface to form a capsule wall (Ji J., Childs R.F. and Mehta M., 2001). Drugs best encapsulated with this method are low-melting solid or poorly soluble organic liquids.

***Electrostatic Method*:**

It is useful when the coating material and drug to be encapsulated are both aerosols and oppositely charged (Zaho Y.,*et al* 2005). The drug and coating material are atomized resulting in the formation of microcapsules, which are then cooled and collected by an appropriate aerosol collecting system.

***Precipitation Process*:**

It covers many techniques. For examples, gelation of calcium alginate with calcium salt solution, and precipitate of a polymer around the drug being encapsulated by adding antisolvent (Tu L.S., Dehghani F. and Foster N.R., 2002).

Hot Melt Techniques:

It involves mechanical drop formation at an elevated temperature followed by a cooling step. The polymers used in this technique have low melt viscosities at

reasonable operating Temperature and can be readily sprayed. Drugs to be encapsulated by this method must be thermally stable (Souto E.B *et al*., 2005).

Salting Out Method:

It involves the addition of salt to an aqueousPolymer solution thereby separating the polymer from solution. One concern with this method is the possibility to incorporate a relatively high concentration of salt into the capsule wall (Konan Y.N., Gurny R. and Allémann E., 2002).

Solvent Evaporation Techniques:

In this method drug and polymer are dissolved in a water immiscible volatile organic solvent and then dispersed into an aqueous solution to form an emulsion, the organic solvent is evaporated and solid microcapsule is formed (Freitas S., Merkle H.P. and Grander B., 2005).

Air Suspension Method:

In this method, the solid-particular core materials are dispersed in a supporting air stream and spray coating the suspended particles with a polymer solution (Rodriguez L.*et al* 2004).

Multiorifice – Centrifuge:

This process is mechanical in nature and use centrifugal forces to core material particles through polymer films formed across a hole in a spinning centrifuge(54), thus enveloping the core-material particles and forming microcapsules. The embryonic

microcapsules are hardened by collection in air or in a solution of a hardening agent (Leon Lachman, Lieberman H A, Kanig J L,).

Pan–Coating Techniques:

The micronized drug material is deposited onto various spherical substrates and coated with multiple layers of film-forming polymers in a coating pan (Pearnchob N and Bodmeier R., 2003).

Spray Drying:

In this method the core material is dispersed into a coating solution or polymer melt and atomized the mixture into an air stream, which is usually heated, the solvent is removed, thus the polymers are collapsed around the particles (Bruschi M.L *et al* 2003).

CHAPTER-II

GASTRO RETENTIVE DRUG DELIVERY SYSTEM

Gastro retentive Drug Delivery System

Gastroretentive dosage forms are drug delivery systems which **remain in the stomach** for an extended period of time and allow both spatial and temporal control of drug liberation. Basically gastroretentive systems swells following ingestion and is retained in the stomach for a number of hours, while it continuously releases the incorporated drug at a controlled rate to preferred **absorption sites** in the upper intestinal tract (Stanley Davies., 2005). Their application can be advantageous in the case of drugs absorbed mainly from the upper part of GIT or are unstable in the medium of distal intestine.

Gastrointestinal Tract

Anatomy of the gastrointestinal tract:

The gastrointestinal tract is divided into three main regions namely:

- Stomach.
- Small intestine (Duodenum, Jejunum and Ileum).
- Large intestine.

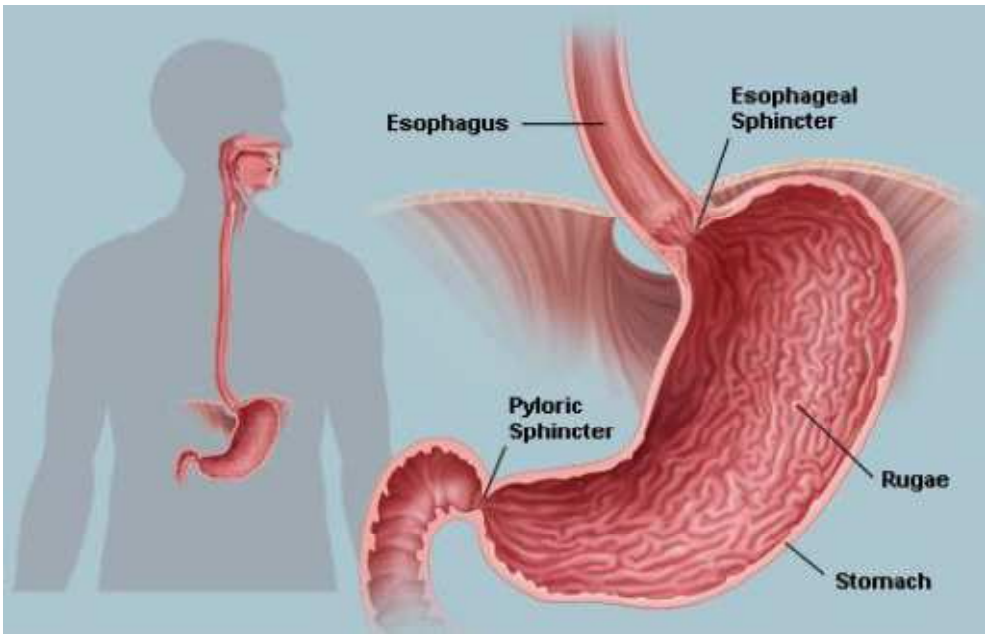


Figure 1

The GIT is a muscular tube, from the mouth to the anus, which functions to take in nutrients and eliminate waste by secretion, motility, digestion, absorption and excretion, which are known as physiological processes. The stomach is a J-shaped enlargement of the GIT which is divided into 4 anatomical regions:

- cardia
- fundus
- body
- antrum.

The main function of the stomach is to store and mix food with gastric secretions before emptying its load (chyme) through the pyloric sphincter and into the small intestine at a controlled rate suitable for digestion and absorption. During empty state, the stomach occupies a volume of about 50 ml, but this may increase to as much as 1 litre when full. The walls of the GIT, from stomach to large intestine, have the

same basic arrangement of tissues, the different layers, from outside to inside, comprising serosa, intermuscular plane, longitudinal muscle, submucosa, circular muscle, lamina propria, muscularis mucosae, and epithelium. In addition to longitudinal and circular muscle, the stomach has a third muscle layer known as the "oblique muscle layer", which is situated in the proximal stomach, branching over the fundus and higher regions of the gastric body. The different smooth muscle layers are responsible for performing the motor functions of the GIT, i.e. gastric emptying and intestinal transit.

Basic gastrointestinal tract physiology

The stomach is divided into 3 regions anatomically: fundus, body, and antrum pylorus. The proximal part is the fundus and the body acts as a reservoir for undigested material, whereas the antrum is the main site for mixing motions and acts as a pump for gastric emptying by propelling actions. Gastric emptying occurs during fasting as well as fed states but the pattern of motility is distinct in the 2 states. During the fasting state an interdigestive series of electrical events take place, which cycle through both stomach and intestine every 2 to 3 hours. This is called the interdigestive myloelectric cycle or migrating myloelectric cycle (MMC), which is divided into following 4 phases are:

- **Phase I:** This period lasts about 30 to 60 minutes with no contractions.
- **Phase II:** This period consists of intermittent contractions that increase gradually in intensity as the phase progresses, and it lasts about 20 to 40 minutes. Gastric discharge of fluid and very small particles begins later in this phase.
- **Phase III:** This is a short period of intense distal and proximal gastric contractions (4-5 contractions per minute) lasting about 10 to 20 minutes these

contractions, also known as ‘‘**house-keeper wave**,’’ sweep gastric contents down the small Intestine.

- **Phase IV:** This is a short transitory period of about 0 to 5 minutes, and the contractions dissipate between the last part of phase III and quiescence of phase

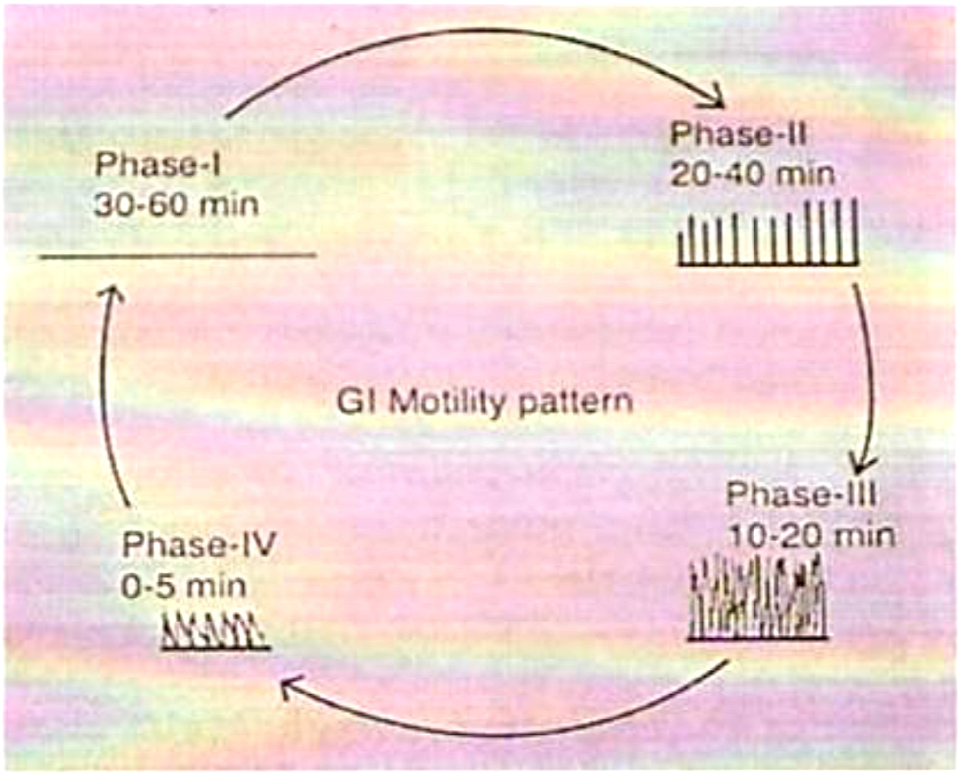


Fig 2 Gastro intestinal motility attterm

Salient features of upper gastrointestinal tract (R. Talukder and R. Fassihi., 2004**)**

Section	Length (m)	Transit time (h)	pH	Microbial count	Absorbing surface area (m^2)	Apsorption pathways
Stomach	0.2	Variable	1-4	$<10^3$	0.1	P,C,A
Small intestine	6-10	3 ± 1	5-7.5	10^3-10^{10}	120-200	P,C,A,F,I,E,CM

P, Passive diffusion; C, Convective or aqueous channel transport; A, Active transport; F, Facilitated transport; I, Ion-pair transport; E, Entero-or pinocytocis; CM, Caveolin Mediated transport.

Need for gastroretention

- Drugs that are absorbed from the **proximal part** of the gastrointestinal tract (GIT).
- Drugs that are **less soluble** or that degrade at the alkaline pH.
- Drugs that are absorbed due to **variable** gastric emptying time.
- Local or sustained drug delivery to the stomach and proximal small intestine to **treat** certain conditions (Brahmankar.D.M., Jaiswal.S.B.,).
- Treatment of **peptic ulcers** caused by H.Pylori infections (Mona Semalty *et al*., 2010).

Formulation considerations for GRDDS

- It must be **effective retention** in the stomach to suit for the clinical demand.
- It must be convenient for intake to facilitate patient compliance.
- It must have sufficient drug loading capacity and control drug release profile.
- It must have full degradation and evacuation of the system once the drug release is over.
- It should not have effect on gastric motility including emptying pattern.
- It should not have other local adverse effects (Vyas, S.P., Khar, R.K.,Targeted and controlled drug deliver,).

Certain types of drugs can benefit from using gastro retentive devices

- Drugs with a **narrow absorption** window.
- Drugs acting **locally** in the stomach.
- Drugs those are primarily absorbed in the stomach.
- Drugs those are **poorly soluble** at an alkaline p^H.
- Drugs absorbed rapidly from the GI tract.
- Drugs those **degrade** in the **colon (**Xiaoling Li, Bhaskara R. Jasti., 2005**)**.

Drugs those are unsuitable for gastro retentive drug delivery systems

- Drugs that have very **limited acid solubility** e.g. Phenytoin etc.
- Drugs that suffer instability in the gastric environment e.g. Erythromycin etc.
- Drugs intended for selective release in the colon e.g. 5- amino salicylic acid and corticosteroids etc. (European Pharmacopoeia 2009).

Factors affecting gastric retention

Various factors that affect the bioavailability of dosage form and efficacy of the gastro retentive system (Xiaoling Li, Bhaskara R. Jasti., 2005**)** are:

- **Density:** Gastric retention time (GRT) is a function of buoyancy of dosage form that is dependent on the density.
- **Size:** Dosage form units with a diameter of more than 7.5 mm are reported to have an increased GRT compared with those with a diameter of 9.9 mm.
- **Shape:** Tetrahedron and ring shaped devices with a flexural modulus of 48 and 22.5 kilo pounds per square inch (KSI) are reported to have better GRT 90% to 100% retention at 24 hours compared with other shapes.
- **Single or Multiple unit formulation:** Multiple unit formulations show a more predictable release profile and insignificant impairing of performance due to

failure of units, allow co-administration of units with different release profiles or containing incompatible substances and permit a larger margin of safety against dosage form failure compared with single unit dosage forms.

- **Fed or unfed state:** Under fasting conditions, the GI motility is characterized by periods of strong motor activity or the migrating myoelectric complex (MMC) that occurs every 1.5 to 2hrs. The MMC sweeps undigested material from the stomach and, if the timing of administration of the formulation coincides with that of the MMC, the GRT of the unit can be expected to be very short. However, in the fed state, MMC is delayed and GRT is considerably longer.
- **Nature of meal:** Feeding of indigestible polymers or fatty acid salts can change the motility pattern of the stomach to a fed state, thus decreasing the gastric emptying rate and prolonging drug release.
- **Caloric content:** GRT can be increased by 4 to 10 hours with a meal that is high in proteins and fats.
- **Frequency of feed:** The GRT can increase by over 400 minutes when successive meals are given compared with a single meal due to the low frequency of MMC.
- **Gender:** Mean ambulatory GRT in males (3.4±0.6 hours) is less compared with their age and race matched female counterparts (4.6±1.2 hours), regardless of the weight, height and body surface).
- **Age:** Elderly people, especially those over 70, have a significantly longer GRT.
- **Posture:** GRT can vary between supine and upright ambulatory states of the patient.

- **Concomitant drug administration:** Anticholinergics like atropine, propantheline, opiates like codeine and prokinetic agents like Metoclopramide and Cisapride, can affect floating time.
- **Biological factors:** Diabetes and Crohn's disease etc.

Approaches to Gastric retention

Various approaches for gastro retentive drug delivery systems are:

(A) Floating drug delivery

(B) Bio/Muco-adhesive systems

(C) Raft-forming systems

(D) Swelling and expanding systems

(E) Superporous Hydrogels

(F) High density systems

(A)Floating drug delivery

Floating Drug Delivery Systems (FDDS) have a bulk **density lower** than gastric fluids and thus remain **buoyant** in the stomach (Bardonnet P.L *et al*., 2006), (Fig.3), for a prolonged period of time, without affecting the gastric emptying rate and the drug is released slowly at a desired rate from the system, results in an increase in the gastric residence time and a better control of fluctuations in the plasma drug concentrations and after complete release of the drug, the residual system is emptied from the stomach.

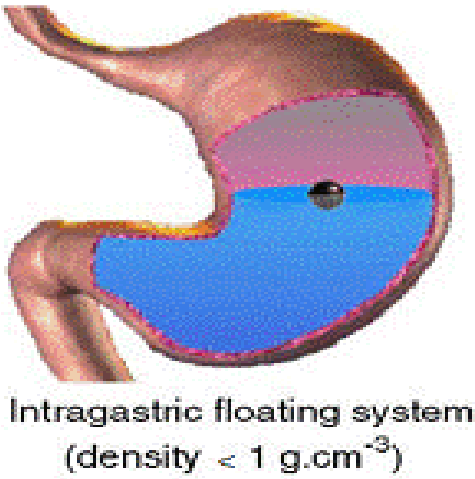


Fig: 3 Graphic of the buoyant tablet which is less dense than the stomach fluid and therefore remains in the fundus.

(B) Bio/Muco-adhesive systems

Bio/muco-adhesive systems, bind to the gastric epithelial cell surface or mucin, which extends the GRT of drug delivery system in the stomachThe surface epithelial adhesive properties of mucin have been well recognized and applied to the development of GRDDS based on bio/muco-adhesive polymers (Fig 4). The ability to provide **adhesion** of a **drug** delivery system to the **gastrointestinal wall** provides longer residence time in a particular organ site, thereby producing an improved effect in terms of local action or systemic effect. Binding of polymers to the mucin/epithelial surface can be divided into three categories:

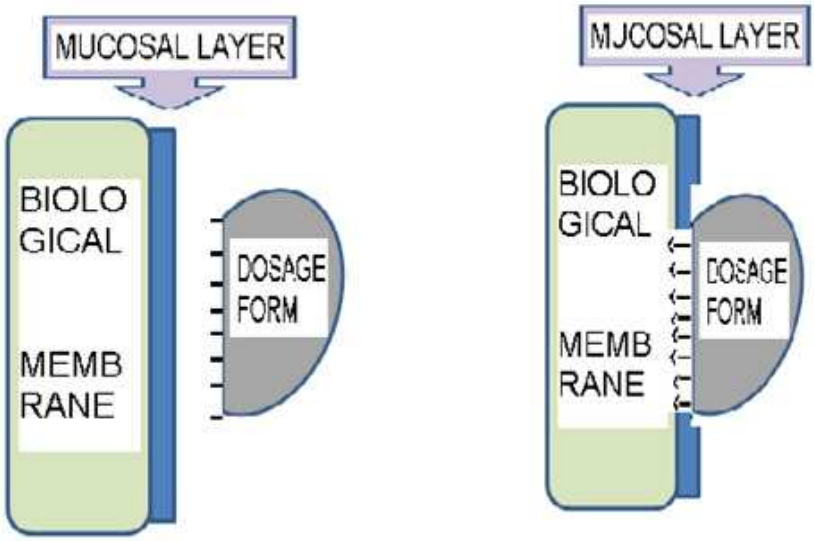


Fig: 4 Bioadhesive systems

(C) Raft-forming systems:

These systems (Bardonnet P.L *et al*., 2006), contain gel-forming solution (e.g. sodium alginate solution containing carbonates or bicarbonates), which on contact with the gastric contents, swells and forms a viscous cohesive gel containing entrapped CO_2 bubbles, releases drug slowly in stomach by forming the raft layer on the top of gastric fluid (Fig.5). These formulations contain antacids such as calcium carbonate or aluminium hydroxide to reduce gastric acidity.

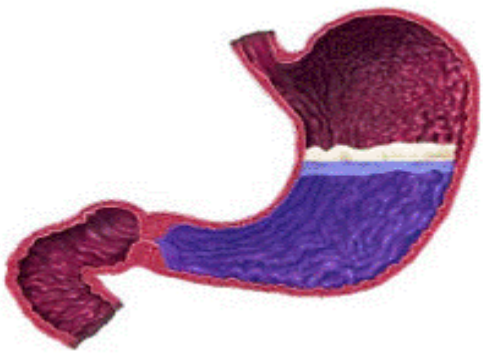


Fig: 5 Barrier formed by a raft-forming system

(D) Swelling and expanding systems:

A dosage form in the stomach will withstand gastric transit if it is bigger than the pyloric sphincter, also the dosage form must be small enough to be swallowed, and must not cause gastric obstruction either singly or by accumulation (Bardonnet P.L *et al*., 2006). Thus, their configurations are required to develop an expandable system in order to prolong the gastric retention time (GRT),

1) A small configuration for oral intake.

2) An expanded gastroretentive form.

3) A final small form enabling evacuation following drug release from the device. Swellable systems, (Fig.6), are also retained in the gastro intestinal tract (GIT) due to their mechanical properties. The swelling is usually results from osmotic absorption of water and the dosage form is small enough to be swallowed by the gastric fluid.

- Expandable systems have some drawbacks like problematical storage of much easily hydrolysable, biodegradable polymers relatively short-lived mechanical shape memory for the unfolding system most difficult to industrialize and not cost effective. Again, permanent retention of rigid, large single-unit expandable drug delivery dosage forms may cause brief obstruction, intestinal adhesion and gastropathy.

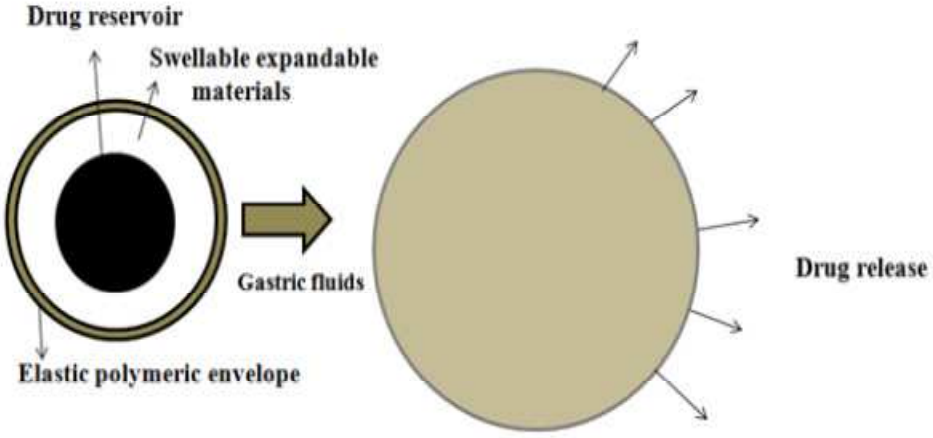


Fig: 6 Drug release from swellable systems

Thus, gastro retentivity is improved by the combination of substantial dimension with high rigidity of dosage form to withstand peristalsis and mechanical contractility of the stomach. Unfoldable and swellable systems have been investigated and recently tried to develop an effective gastro retentive drug delivery.

Unfoldable and swellable systems have been investigated and recently tried to develop an effective gastro retentive drug delivery.

Unfoldable systems are made of biodegradable polymers. They are available in different **geometric forms**, like tetrahedron, ring or planner membrane (4 - label disc or 4 - limbed cross form) of bioerodible polymer compressed within a capsule which extends in the stomach.

(E) Superporous Hydrogels:

Conventional hydrogels, with pore size ranging between **10 nm and 10 µm** has very slow process of water absorption and require several hours to reach an equilibrium state during which premature evacuation of the dosage form may occur while the superporous hydrogel (Bardonnet P.L *et al*., 2006), (Fig.7), having average pore size

(>100 μm), swell to equilibrium size within a minute, due to **rapid water uptake by capillary wetting** through numerous interconnected open pores. Moreover they swell to a large size (swelling ratio 100 or more) and are intended to have sufficient mechanical strength to withstand pressure by gastric contractions. This is achieved by a co-formulation of a hydrophilic particulate material, Ac-Di-Sol (Croscarmellose sodium).

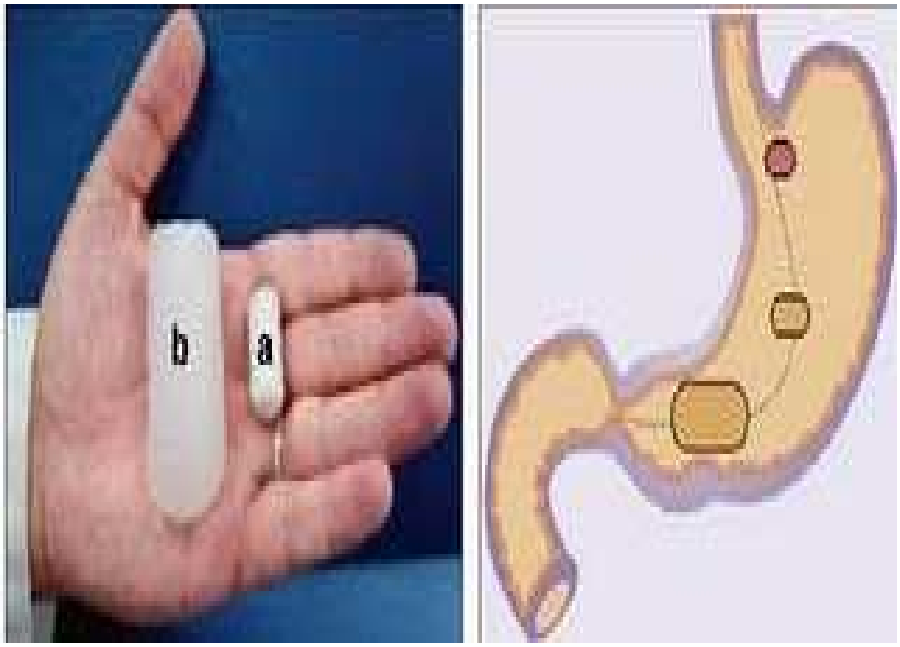


Fig:7 On the left, Superporous Hydrogels in its dry (a) and water-swollen (b) state. On the right, schematic illustration of the transit of Superporous Hydrogel.

(F) Magnetic systems:

This approach is based on the simple principle that the dosage form contains a small **internal magnet**, and a magnet placed on the abdomen over the position of the stomach to enhance the gastric retention time (GRT) (Bardonnet P.L *et al*., 2006),. The external magnet must be positioned with a degree of high precision that might compromise patient compliance.

(G) Self-unfolding systems:

The self-unfolding systems are capable of mechanically increasing in size relative to the initial dimensions. This increase prevents the system from passing through the pylorus and retains for a prolonged period of time in the stomach. A drug can be either contained in a polymeric composition of the gastro retentive system or included as a separate component. Several methods, were suggested to provide for the self-unfolding effect

- The use of hydrogels swelling in contact with the gastric juice.
- **Osmotic systems**, comprising an osmotic medium in a semi-permeable membrane
- Systems based on low-boiling liquids converting into a gas at the body temperature

(H) High density systems:

These systems with a density of about 3 g/cm^3 are retained in the rugae of the stomach and are capable of withstanding its peristaltic movements. A density of **2.6-2.8 g/cm^3** acts as a threshold value after which such systems can be retained in the lower part of the stomach. High density formulations include coated pellets. Coating is done by heavy inert material such as barium sulphate, zinc oxide, titanium dioxide, iron powder etc. They are retained in the antrum of stomach, (Fig.8).

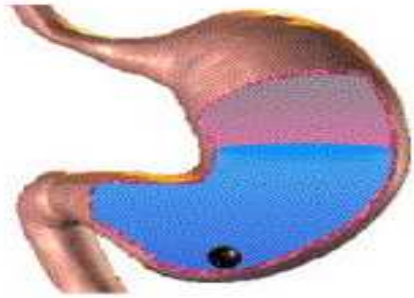


Fig: 8 Graphic of heavy tablet which is denser than the stomach fluid and therefore sinks to the antrum

Floating drug delivery systems:

A floating dosage form is useful for drugs acting locally in the proximal gastrointestinal tract. These systems are also useful for drugs that are poorly soluble (or) unstable in intestinal fluids. The floating properties of these systems help to retain in the stomach for a long time. Various attempts have been made to develop floating systems, which float on the gastric contents and release drug molecules for the desired time period. After the release of a drug, the remnants of the system are emptied from the stomach.

Based on the mechanism of buoyancy, two different technologies have been used in development of floating drug delivery systems. These include:

a) Effervescent system.

b) Non- Effervescent system.

a) Effervescent Systems

Effervescent systems, include use of gas generating agents, carbonates (e.g. Sodium bicarbonate) and other organic acid (e.g. citric acid and tartaric acid) present in the formulation to **produce carbon dioxide (CO_2)** gas, thus reducing the density of the system and making it float on the gastric fluid. An alternative is the incorporation of matrix containing portion of liquid, which produce gas that evaporate at body temperature

.

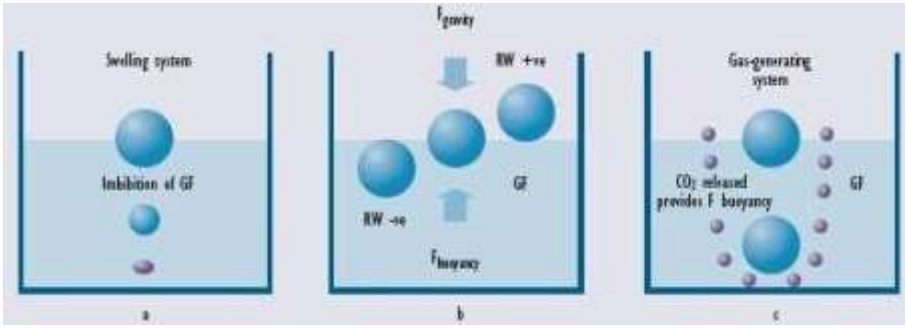


Fig: 9 Gas generating systems

These effervescent systems further classified into two types:

1) Gas generating systems.
2) Volatile liquid or vacuum containing systems.

1) Gas generating systems

A) Tablets:

1. Intragastric single layer floating tablets or Hydrodynamically Balanced System (HBS)

These formulations have bulk density lower than gastric fluids and thus float in the stomach that increases the gastric emptying rate for a prolonged period, (Fig.10). These are formulated by intimately mixing the gas (CO_2) generating agents and the drug within the matrix tablet. The drug is released slowly at a desired rate from the floating system and the residual system is emptied from the stomach after the complete release of the drug. This leads to an increase in the gastric residence time (GRT) and a better control over fluctuations in plasma drug concentration.

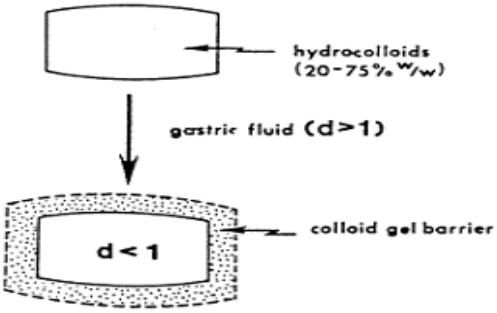


Fig 10 Intragastric single layer floating tablet

2. Intragastric bilayer floating tablets

These are also compressed tablets, containing two layers (Fig.11):

- Immediate release layer
- Sustained release layer.

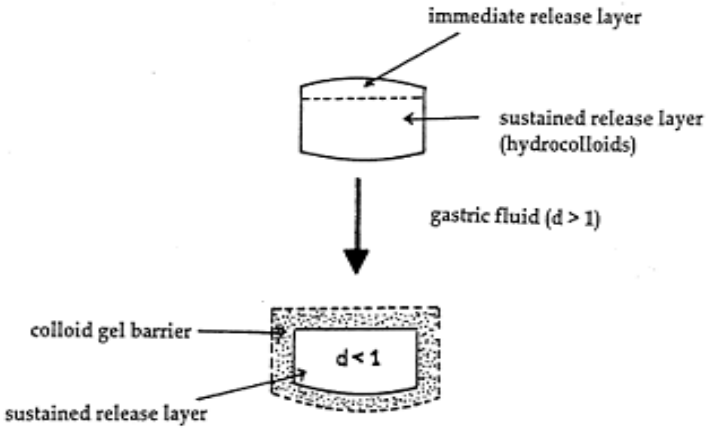


Fig 11: Intragastric bilayer floating tablet

B) Floating capsules

These floating capsules are formulated by filling with a mixture of sodium alginate and sodium bicarbonate. The systems float as a result of the generation of CO_2 that was trapped in the hydrating gel network on exposure to an acidic environment.

C) Multiple unit type floating pills

These multiple unit type floating pills are sustained release pills, known as 'seeds', which are surrounded by two layers. The outer layer is of swellable membrane layer while the inner layer consists of effervescent agents. This system sinks at once and then it forms swollen pills like balloons which float as they have lower density, when it is immersed in the dissolution medium at body temperature. The lower density is due to generation and entrapment of CO_2 within the system.

D) Floating system with Ion-Exchange resins

Floating system using bicarbonate loaded ion exchange resin was made by mixing the beads with **1M sodium bicarbonate** solution, and then the semi-permeable membrane is used to surround the loaded beads to avoid sudden loss of CO_2. On contact with gastric contents an exchange of bicarbonate and chloride ions takes place that

results in generation of CO_2 that carries beads towards the top of gastric contents and producing a floating layer of resin beads.

2) Volatile liquid or vacuum containing systems

(a) Intragastric floating gastrointestinal drug delivery system

This system floats in the stomach because of floatation chamber, which is **vacuum** or filled with a harmless gas or air, while the drug reservoir is **encapsulated** by a microporous compartment.

(b) Inflatable gastrointestinal delivery systems

These systems are incorporated with an inflatable chamber, which contains liquid ether that **gasifies at body temperature** to inflate the chamber in the stomach. These systems are fabricated by loading the inflatable chamber with a drug reservoir, which can be a drug, impregnated polymeric matrix, then encapsulated in a gelatine capsule. After oral administration, the capsule dissolves to release the drug reservoir together with the inflatable chamber. The inflatable chamber automatically inflates and retains the drug reservoir compartment in the stomach. The drug is released continuously from the reservoir into gastric fluid.

c) Intragastric osmotically controlled drug delivery system

This system is comprised of an osmotic pressure controlled drug delivery device and an inflatable floating support in a biodegradable capsule, On contact with the gastric contents in the stomach, the capsule disintegrates quickly to release the intragastirc osmotically controlled drug delivery device. The inflatable support inside forms a hollow polymeric bag which contains a liquid that gasifies at body temperature

to inflate the bag and it is deformable. The osmotic pressure controlled drug delivery device consists of two components, **osmotically active** compartment and a drug reservoir compartment. The drug reservoir compartment is enclosed by a pressure responsive collapsible bag, which is impermeable to liquid and vapour and has a drug delivery orifice. The osmotically active compartment contains an osmotically active salt and is enclosed within a semi-permeable housing. In the stomach, the osmotically active salt present in the osmotically active compartment is dissolved by absorbing the water continuously present in the GI fluid through the semi-permeable membrane. An osmotic pressure is thus created which acts on the collapsible bag and in turn forces the drug reservoir compartment to reduce its volume and activate the drug reservoir compartment to reduce its volume and activate the drug release of a drug solution formulation through the delivery orifice. The floating support is also made to contain a bioerodible plug that erodes after a predetermined time to deflate the support. The deflated drug delivery system is then emptied from the stomach.

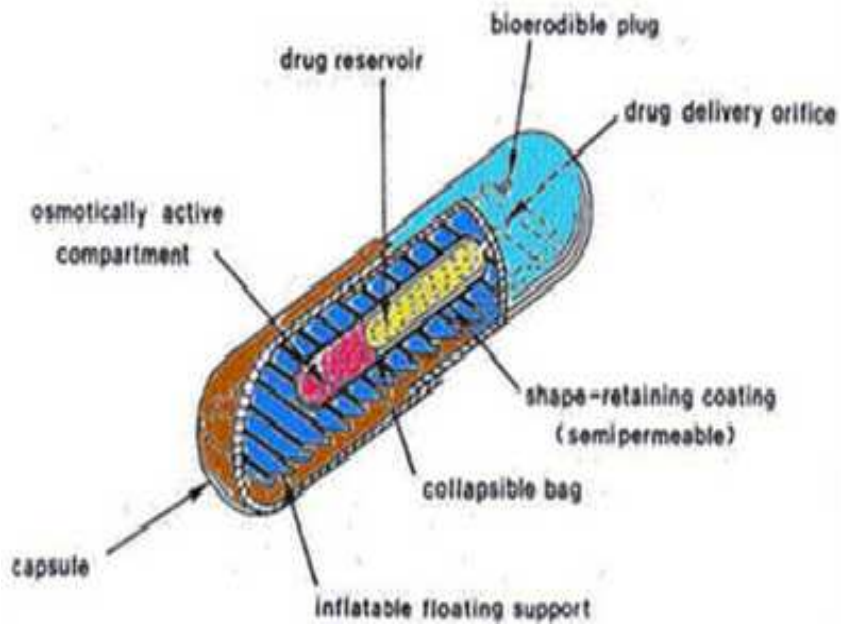


Fig: 12 Intragastric osmotically controlled drug delivery system

b) Non-Effervescent systems

The Non-Effervescent floating drug delivery systems are based on mechanism of swelling of polymer or bioadhesion to mucosal layer in GI tract. The various types of this system are:

1) Single layer floating tablets:

These are formulated by intimate mixing of drug with a gel forming hydrocolloid, that swells on contact with gastric fluid and maintain bulk density of less than unity. The air trapped by the **swollen polymer** confers buoyancy to these dosage forms.

2) Bilayer floating tablets

A bilayer tablet contain **two layer** one immediate release layer which release initial dose from system while the another sustained release layer absorbs gastric fluid, forming an impermeable colloidal gel barrier on its surface, and maintain a bulk density of less than unity and thereby it remains buoyant in the stomach.

3) Alginate beads

Multi unit floating dosage forms were developed from freeze dried calcium alginate. Spherical beads of approximately 2.5 mm diameter can be prepared by dropping a sodium alginate solution into aqueous solution of $CaCl_2$, causing precipitation of calcium alginate leading to formation of **porous** system, which can maintain a floating force for over 12 hours. When compared with solid beads, which gave a short residence, time of 1 hr, and these floating beads gave a prolonged residence time of more than 5.5 hours.

4) **Hollow microspheres**

Hollow microspheres (microballons), loaded with drug in their outer polymer shells were prepared by a novel emulsion-solvent diffusion method (Xiaoling Li,

Bhaskara R. Jasti, 2005) (Fig.13). The ethanol: dichloromethane solution of the drug and an enteric acrylic polymer was poured into an agitated aqueous solution of PVA that was thermally controlled at 40^0C. The gas phase generated in dispersed polymer droplet by evaporation of dichloromethane formed an internal cavity in **microsphere of polymer with drug.** The microballons floated continuously over the surface of acidic dissolution media containing surfactant for more than 12 hours *in vitro* (Alexander Streubel *et al*., 2006).

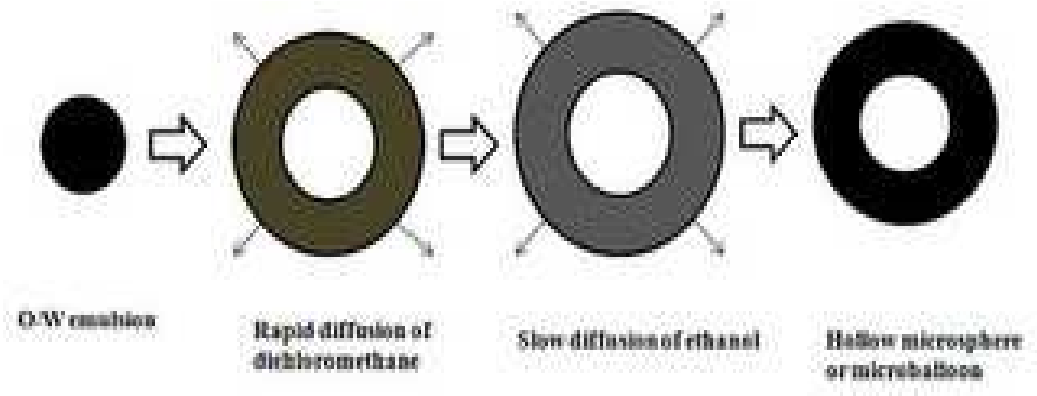


Fig: 13 Formulation of floating hollow microsphere or microballoon

EVALUATION OF FLOATING DOSAGE FORMS

1. **For Single Unit Dosage Forms (ex: tablets**). (Baumgartner S *et al*.,2000 & Rosa M. *et al.*,1994)

(i) Floating lag time: It is the time taken by the tablet to emerge onto the surface of dissolution medium and is expressed in seconds or minutes.

(ii) Invitro drug release and duration of floating: This is determined by using USP II apparatus (paddle) stirring at a speed of 50 or 100 rpm at 37 ± 0.2 °c in simulated gastric fluid (pH 1.2 without pepsin). Aliquots of the samples are collected and

analysed for the drug content. The time (hrs) for which the tablets remain buoyant on the surface of the dissolution medium is the duration of floating and is visually observed.

(iii) In vivo evaluation for gastro-retention: This is carried out by means of X-ray or Gammascintigraphic monitoring of the dosage form transition in the GIT. The tablets are also evaluated for hardness, weight variation, etc.

2. For Multiple Unit Dosage Forms (ex: microspheres) Apart from the In vitro release, duration of floating and in vivo gastro-retention tests, the multiple unit dosage forms are also evaluated for

(i) ***Morphological and dimensional analysis*** with the aid of scanning electron microscopy (SEM). The size can also be measured using an optical microscope.

(ii) ***% yield of microspheres:*** This is calculated from Weight of microspheres obtained ×100 / total weight of drug

and polymer

(iii) ***Entrapment efficiency:*** The drug is extracted by a suitable method, analysed and is calculated from

Practical amount of drug present ×100 / Theoretical drug content

(***iv) In vitro floating ability (Buoyancy %):*** A known quantity of microspheres are spread over the surface of a USP (Type II) dissolution apparatus filled with 900 ml of 0.1 N HCl containing 0.002% v/v Tween 80 and agitated at 100 rpm for 12 hours. After 12 hours, the floating and settled layers are seperated, dried in a dessicator and weighed.

The buoyancy is calculated from the following formula.

$$\text{Buoyancy (\%)} = W_f / (W_f + W_s) * 100$$

Where, W_f and Ws are the weights of floating and settled microspheres respectively.

(v) Drug-excipient (DE) interactions: This is done using FTIR. Appearance of a new peak, and/or disappearance of original drug or excipient peak indicate the DE interaction. Apart from the above mentioned evaluation parameters, granules (ex: Gelucire 43/01) are also evaluated for the effect of ageing with the help of Differential Scanning Calorimeter or Hot stage polarizing microscopy.

CHARACTERIZATION PARAMETERS:

1. ***Size and shape evaluation:***

The particle size and shape plays a major role in determining solubility rate of the drugs and thus potentially its bioavailability. The particle size of the formulation was determined using Sieve analysis, Air elutriation analysis, Photo analysis, Optical microscope ,Electro résistance counting methods (Coulter counter), Sedimentation techniques, Laser diffraction methods, ultrasound attenuation spectroscopy, Air Pollution Emissions Measurements etc.

2. ***Floating Properties:***

Effect of formulation variables on the floating properties of gastric floating drug delivery system was determined by using continuous floating monitoring system and statistical experimental design.

3. ***Surface Topography:***

The surface topography and structures were determined using scanning electron microscope (SEM, JEOL JSM – 6701 F, Japan) operated with an acceleration voltage of 10k.v, Contact angle meter, Atomic force microscopy (AFM), Contact profiliometer.

4. ***Swelling Studies****:*

Swelling studies were performed to calculate molecular parameters of swollen polymers. Swelling studies was determined by using Dissolution apparatus, optical microscopy and other sophisticated techniques which include H1NMRimaging, Confocal laser scanning microscopy (CLSM), Cryogenic scanning electron microscopy (Cryo-SEM), Light scattering imaging (LSI) etc. The swelling studies by using Dissolution apparatus (USP dissolution apparatus (usp-24) labindia disso 2000) was calculated as per the following formula. (Ferdous Khan *et al*., 2008)

Swelling ratio = Weight of wet formulation / Weight offormulations

5. Determination of the Drug Content:

Percentage drug content provides how much amount of the drug that was present in the formulation. It should not exceed. the limits acquired by the standard monographs. Drug content was determined by using HPLC, HPTLC methods, near infrared spectroscopy (NIRS), Microtitrimetric methods, Inductively Coupled Plasma Atomic Emission Spectrometer (ICPAES) and also by using spectroscopy techniques.

6. Percentage Entrapment Efficiency:

Percentage entrapment efficiency was reliable for quantifying the phase distribution of drug in the prepared formulations. Entrapment efficiency was determined by using three methods such as Micro dialysis method, Ultra centrifugation, and pressure Ultra filtration.

7. In-vitro Release Studies:

In vitro release studies (USP dissolution apparatus (USP- 24) lab India disso 2000) were performed to provide the amount of the drug that is released at a definite time period. Release studies were performed by using Franz diffusion cell system and synthetic membrane as well as different types of dissolution apparatus.

8. Powder X-ray Diffraction:

X-ray powder diffraction (Philips analytical, modelpw1710) is the predominant tool for the study of poly-crystalline materials and is eminently suited for the routine characterization of pharmaceutical solids. Samples were irradiated with α radiation and analyzed between 2 ºC and 60 ºC .The voltage and current used were 30KV and 30mA respectively.

9. Fourier Transform Infrared Analysis:

Fourier transform infrared spectroscopy (FT-IR, Shimadzu, Model-RT-IR-8300) is a technique mostly used to identify organic, polymeric, and some inorganic materials as well as for functional group determination. Fourier Transform Infrared Analysis (FT-IR) measurements of pure drug, polymer and drug loaded polymer formulations were obtained on FTIR. The pellets were prepared on KBr-press under hydraulic pressure of 150kg/cm2; the spectra were scanned over the wave number range of 3600 to 400 cm-1 at the ambient temperature.

10. Differential Scanning Calorimetric (DSC):

DSC (Shimadzu, Model-DSC-60/DSC-50/ Metler Toldeo) are used to characterize water of hydration of pharmaceuticals .Thermo grams of formulated preparations were obtained using DSC instrument equipped with an intercooler. Indium/Zinc standards

were used to calibrate the DSC temperature and enthalpy scale. The sample preparations were hermitically sealed in an aluminium pan and heated at a constant rate of 10°C/min; over a temperature range of 25° C – 65°C. Inert atmosphere was maintained by purging nitrogen gas at the flow rate of 50ml/min.

III) IN-VIVO EVALUATION

a) ***Radiology***

X-ray is widely used for examination of internal body systems. Barium Sulphate is widely

used Radio Opaque Marker. So, $BaSO_4$ is incorporated inside dosage form and X-ray images are taken at various intervals to view the dosage form.

b*) Scintigraphy*

Similar to X-ray, emitting materials are incorporated into dosage form and then images are

taken by scintigraphy. Widely used emitting material is 99Tc.

c) Gastroscopy

Gastroscopy is peroral endoscopy used with fibre optics or video systems. Gastroscopy is used to inspect visually the effect of prolongation in stomach. It can also give the detailed evaluation of GRDDS.

d) Magnetic Marker Monitoring

In this technique, dosage form is magnetically marked with incorporating iron powder inside, and images can be taken by very sensitive bio-magnetic measurement equipment. Advantage of this method is that it is radiation less and so not hazardous.

e) Ultrasonography

Used sometimes, not used generally because it is not traceable at intestine.

Advantages of floating drug delivery system

- The principle of Hydrodynamically Balanced System (HBS) can be used for any particular medicament or class of medicament. The HBS formulations are not restricted to medicaments, which are principally absorbed from the stomach, since it has been found that these are equally efficacious with medicaments which are absorbed from the intestine. E.g. Chlorpheniramine maleate.

- The HBS are advantageous for drugs absorbed through the stomach e.g. ferrous salts and for drugs meant for local action in the stomach and treatment of peptic ulcer disease e.g. antacids.

- The efficacy of the medicaments administered utilizing the sustained release principle of HBS has been found to be independent of the site of absorption of the particular medicaments.

- Administration of a prolonged release floating dosage form tablet or capsule will result in dissolution of the drug in gastric fluid. After emptying of the stomach contents, the dissolved drug is available for absorption in the small intestine, therefore it is expected that a drug will be fully absorbed from the floating dosage form if it remains in solution form even at alkaline p^H of the intestine.
- Many drugs categorized as once-a-day delivery have been demonstrated to have suboptimal absorption due to dependence on the transit time of the dosage form, making traditional extended release development challenging. Therefore,

a system designed for longer gastric retention will extend the time within which drug absorption can occur in the small intestine.

- When there is vigorous intestinal movement and a short transit time as might occur in certain type of diarrhoea, poor absorption is expected under such circumstances it may be advantageous to keep the drug in floating condition in stomach to get a relatively better response.
- Gastric retention will provide advantages such as the delivery of drugs with narrow absorption windows in the small intestinal region.

Limitations of floating drug delivery system

- The floating system requires, sufficiently high level of fluid in the stomach for the system to float, this can be overcome by administering dosage form with a glass full of water (200-250 ml) or coating the dosage form with bioadhesive polymer which adhere to gastric mucosa.
- Aspirin and non-steroidal anti-inflammatory drugs are known to cause gastric lesions, and slow release of such drugs in the stomach is unwanted.
- Drugs, such as Isosorbide dinitrate, that are absorbed equally throughout the GI tract, drugs undergoing first pass metabolism will not benefit from incorporation into a gastric retention system.
- Floating dosage form should not be given to the patients just before going to the bed as gastric emptying occurs rapidly when the subject remains in supine posture.
- Drugs that have stability or solubility problem in gastrointestinal fluid or that irritate gastric mucosa are not suitable.

- Drugs that have multiple absorption sites or which undergo first pass metabolism were not desirable.
- The single unit floating dosage form is associated with "all or none concept". This problem can be overcome by formulating multiple unit system like floating microballoons or microspheres.

Applications of floating drug delivery system

Sustained drug delivery:

Hydrodynamically Balanced System (HBS) type are dosage forms which have bulk density less than one, relatively large in size and did not easily pass through pylorus, releases the drug over a **prolonged period of time** by retaining in the stomach for several hours and by increasing the gastric residence time (Manoj Goyal *et al*., 2011).

Site specific drug delivery:

Floating drug delivery systems are particularly useful for drugs having **specific absorption** from stomach or proximal part of the small intestine e.g. riboflavin, furosemide etc. The absorption of captopril has been found to be site specific, stomach being the major site followed by duodenum (Manoj Goyal *et al*., 2011)..

***Absorption enhancement*:**

Drugs that have **poor bioavailability**, because of their absorption is restricted to upper GIT are potential candidates to be formulated as floating drug delivery systems, thereby improving their absolute bioavailability (Manoj Goyal *et al*., 2011)..

Minimized adverse activity at the colon

Retention of the drug at the stomach (HBS system), minimizes the amount of drug that reaches the colon, that **prevents the undesirable** activities of the drug in colon (Manoj Goyal *et al*., 2011). This Pharmacodynamic aspect provides the rationale for GRDF formulation for betalactam antibiotics that are absorbed only from the small intestine, and whose presence in the colon leads to the development of microorganism's resistance.

Reduction in plasma fluctuations*:*

Patients with advanced Parkinson's disease, experienced pronounced fluctuations in symptoms while treatment with standard L-dopa. A HBS dosage form provided a better control of motor fluctuations although its bioavailability was reduced by 50-60% of the standard formulation (Manoj Goyal *et al*., 2011).

Peptic ulcer treatment**:**

H. Pylori, causative bacterium for peptic ulcers and chronic gastritis. Patients require high concentration of drug, to be maintained at the site of infection that is within the gastric mucosa. The floating dosage form due to its floating ability was retained in stomach and maintained high concentration of drug in the stomach. A sustained liquid preparation of Ampicillin, using sodium alginate was developed that spreads out and **adheres to gastric mucosal** surfaces and releases the drug continuously.

Suitable for poorly absorbed drugs*.*

Floating drug delivery systems are particularly useful for drugs which are poorly soluble or unstable in intestinal fluids and acid stable drugs and for those which undergo abrupt changes in their pH-dependent solubility due to pathophysiological conditions of GIT, food and age, e.g. floating system for furosemide lead to potential treatment of Parkinson's disease. Approximate 30% drug was absorbed after oral administration.

CHAPTER-III

LITERATURE REVIEW

Anand Gadad *et al*., 2011, developed and evaluated floating microspheres of captopril to prolong its gastric residence time in stomach. Floating microspheres was formulated using bio compatible polymers like Eudragit S100 and Ethyl cellulose in different proportion by solvent evaporation technique. The microsphere with Ethlcellulose showed higher buoyancy when compared with Eudragit S100 polymer.

Gangadharappa H. V *et al*., 2011, developed and evaluated Hollow Microspheres of Rosiglitazone Maleate, Microspheres were prepared by modified Quasi-emulsion diffusion technique using ethyl cellulose, eudragit S100, polyethylene oxide and Hydroxyproply methyl cellulose (HPMC K15M) as polymers. The formulations were evaluated for micromeritic properties, in vitro, in vivo buoyancy, % yield, entrapment efficiency, in vitro and in vivo release studies. SEM photographs showed the outer surface of microspheres was smooth and dense whereas internal surface was porous which helped to prolong floating to increase residence time in stomach. All the formulations floated for more than 8 h. In vitro drug release studies showed controlled release of rosiglitazone maleate for over 12 h. The release behaviour best fitted mostly in peppas and zero order equations. In vivo evaluation of blood glucose levels in albino rats showed that floating microspheres of rosiglitazone maleate had better glycemic control than conventional dosage form.

Mallikarjuna Rao K *et al*., 2011, prepared and evaluated floating microspheres with Amoxycillin trihydrate as a model drug for prolongation of gastric residence time. The microspheres were prepared by the Non-aqueous solvent diffusion method using polymers HPMC and Ethyl cellulose. By the observation of all formulation results concluded that D: Polymer (1:2), polymer Ec:HPMC (1.5gm:0.5gm) containing formulation showed the better drug release.

Kumar Darapu B N *et al*., 2011, formulated and evaluated Gastroretensive Floating Microspheres of Ranitidine Hydrochloride by using HPMC K 100, Xanthan gum and Eudragit S-100 and in various ratios of 1: 1, 1: 2, and 1: 3. The formulations were evaluated for FTIR, drug loading, % entrapment, particle size, SEM, buoyancy, dissolution study and the drug release kinetics. Comparison of three polymers revealed HPMC to be a suitable candidate for sustained release.

M.Saravanan, B.Anupama., 2011, developed and evaluated Ethyl cellulose and PEG blend floating microspheres loaded with ranitidine hydrochloride by novel solvent evaporation matrix erosion method. PEG employed as pore forming agent to induce buoyancy. The drug loaded microsphere could float for 10 hrs and sustain the drug release over 4 to 6 hrs.

Yuanfen Liu *et al*., 2011, produced hollow and bio adhesive microspheres to lengthen drug retention time in the stomach. In these microspheres, ethyl cellulose was used as the matrix, Eudragit EPO was employed to modulate the release rate,

and glyceryl monooleate (GMO) was the bio adhesive polymer insitu. Pharmacokinetic analysis indicated that the elimination half-life time of the hollow bio adhesive microspheres was prolonged and that the elimination rate was decreased.

Amol V. Pande *et al*., 2010, done a project on in-vitro and in-vivo evaluation of ethyl cellulose based floating microspheres of cepodoxime proxetil. GRDDS for cepodoxime proxetile using EC & HPMC as a release retarded material by solvent evaporation technique. In-vivo studies revealed that the relative bioavailability of the drug increased by more than 1.5 times by formulating in to microspheres. From the result it can be concluded that biocompatible and cost effective polymer like EC can be used to formulate an efficient floating micro particulate system with good percentage Entrapment efficiency and practical yield.

Anitha Kakkaerle *et al.*, 2010, developed to prolong gastric residence time by floating matrix tablets of Alfuzosin hydrochloride. The tablets were prepared by direct compression and melt granulation technique, using polymers such as HPMC K15, SCMC, Compritol 888 ATO and either alone or in combination, and other excipients. Prepared tablets were evaluated in-vitro as well as in vivo studies. In-vivo studies showed that the tablets retained in stomach for 6 hours. It was concluded that. HPMC K15 alone retarded the drug release for highly water soluble drug for period of 12 hours.

C.Sharon Kumar *et al*., 2010, done a project on formulation and evaluation of floating microspheres of Gabapentin by using solvent evaporation method using different ratio of sodium alginate polymer. The increase of polymer concentration the drug release was retard. The release kinetics of gabapentin followed super case II transport diffusion.

Ghodake J.D *et al*., 2010, done a project on formulation and evaluation of floating microspheres containing Anti-diabetic (Metformin hydrochloride) drug, the floating microspheres prepared by the emulsion solvent diffusion technique using polymers HPMC K4M and Eudragit RS100. The developed floating microsphere of Metformin hydrochloride may be used in clinic for prolonged drug release in stomach for at least 8hrs, thereby improving the bioavailability and patient compliance.

M.Najmuddin *et al*., 2010, developed floating microspheres of ketoprofen were repared by solvent evaporation method using HPMC and two different grades of Ethyl cellulose as a polymer. The HPMC & Ethyl cellulose which exhibit excellent percentage yield, in-vitro buoyancy, Entrapment efficiency and drug release 98.88% for a period of 12 hrs.

Mona Semaity *et al*., 2010, prepared and characterized gastroretentive floating microspheres of ofloxacin hydrochloride. Formulation of ofloxacin Hcl were prepared as floating microsphere by solvent diffusion technique using polymers

such as EC, PVP K90 and PVA in different ratio. In-vitro floatability studies revealed that most of the microspheres (52.5% to 95.5%) were floatable. It was concluded that these floating microsphere can be selected for the development of gastroretentive drug delivery system of ofloxacin Hcl for potential therapeutic uses.

Najmuddin. M *et al*., 2010, developed and evaluated floating microspheres of ketoprofen, Ketoprofen is NSAID drug with short elimination half-life. Floating microspheres of ketoprofen were prepared by solvent evaporation method using HPMC and two different grades of Ethyl cellulose polymers. The floating microspheres were evaluated such as micromeritic properties, percentage yielg, particle size, drug content determination, Encapsulation efficiency, *In-vitro* buoyancy *In-vitro* drug release, drug compatibily studies, and SEM studies. The results showed that as the concentration of polymer increased it affects the particle size, percentage yield, *in-vitro* buoyancy and drug release of microsphere. The results of these studies suggest that floating microspheres of ketoprofen can be successfully designed to develop sustained drug delivery which can reduce dosing frequency.

Pandey Manisha *et al*., 2010, formulated and evaluated floating microspheres of famotidine. Floating microspheres were prepared by solvent evaporation method using HPMC and Ethyl cellulose as a rate controlling polymer. The developed formulation showed prolonged drug release in stomach for at least 12hrs.

Rajeev Garg and GD Gupta., 2010, prepared and evaluated floating microspheres of silymarin for prolonged gastric residence time and increased drug bioavailability. The work concluded tha the prepared formulation with a suitable blend of Eudragit

S with Eudragit RL and HPMC with Ethyl cellulose, demonstrated satisfactory release and floating properties. Drug release from the formulation followed zero order kinetics and the mechanism of drug release was diffusion controlled.

Sharon Kumar. C *et al.*, 2010, was prepared and characterized floating microspheres of gabapentin by using the solvent evaporation method. The prepare microspheres were evaluated for different evaluation parameters such as percentage yielg, particle size, drug content determination, Encapsulation efficiency, *In-vitro* drug release. Increased in polymer concentration the drug release was controlled. The release kinetics of gabapentin followed supercase II transport diffusion.

Chudiwal P.D *et al*., 2009, developed and optimized gastroretentive drug delivery system of clarithromycin floating microspheres by the optimization technique. The clarithromycin microspheres prepared by non-aqueous solvent evaporation method using different grades of HPMC such as HPMC 15M, HPMC K4M, HPMC 100LV and Ethyl cellulose. The method for preparation of floating microspheres of clarithromycin with optimal yield, Entrapment Efficiency and buoyancy as well as optimal release properties was determined using experimental design methodology.

Gattani Y. S *et al*., 2009, Formulated and evaluated Gastro retentive Multiparticulate Drug delivery system of Aceclofenac by the emulsification solvent-evaporation technique consisting of eudragit RS 100 as a polymer. Effects of polymer concentration, stirring rate during preparation and effect of temperature

on size and drug release was evaluated. The prepared microspheres exhibited prolonged drug release (> 12h) and remained buoyant for > 12 h. The mean particle size increased and the drug release rate decreased at higher polymer concentration. In-vitro studies demonstrated diffusion controlled drug release from the microspheres.

Madan Mohan Kamila *et al*., 2009, developed Multiunit Floating Drug Delivery System of Rosiglitazone Maleate. By encapsulating the drug into Eudragit® RS100 through nonaqueous emulsification/solvent evaporation method. The in vitro performances of microspheres were evaluated by yield (%), particle size analysis, drug entrapment efficiency, in vitro floating behavior, surface topography, drug–polymer compatibility, crystallinity of the drug in the microspheres, and drug release studies. The results showed that floating microspheres could be successfully prepared with good yields (69–75%), high entrapment (78-97%), narrow size distribution, and desired target release. In vivo evaluation in albino rats suggested that floating microspheres of rosiglitazone could be a promising approach for better glycemic control.

Ramji Anil Kumar Arza *et al*., 2009, Formulated and Evaluated Swellable and Floating Gastroretentive Ciprofloxacin Hydrochloride Tablets using a combination of hydrophilic polymer (hydroxypropyl methylcellulose), swelling agents (crospovidone, sodium starch glycolate, and croscarmelose sodium) and effervescent substance (sodium bicarbonate). Formulations are evaluated for percentage swelling, in vitro drug release, floating lag time, total duration of

floating, and mean residence time (MRT) in the stomach. The drug release of optimized formulation follows the Higuchi kinetic model, and the mechanism is found to be non-Fickian/anomalous according to Krosmeyer–Peppas (n value is 0.68). A combination of HPMC K100M, crospovidone, and sodium carbonate shows the good swelling, drug release, and floating characters than the CIFRAN OD® (marketed product).

V.S.Mastiholimath *et al*., 2008, done a project on invitro and invivo evaluation of ranitidine hydrochloride Ethyl cellulose floating microparticles. The formulated microspheres was done by solvent evaporation technique with modification by using an Ethanol co-solvent system. From the experimental results it can be concluded that biocompatible polymers like Ethyl cellulose can be used to formulate an efficient flating microparticulate system with good percentage Entrapment Efficiency and practical yield.

Yasunori Miyazalei *et al*., 2008, done a project on Comparison of gastroretentive microspheres and sustained release preparations using theophylline Theophylline pharmacokinetic studies were conducted in Beagle dogs, comparing bulk powder, commercial sustained-release granules (Theodur™), sustained-release microspheres, floatable microspheres and mucoadhesive microspheres. Overall, the gastroretentive microspheres improved the extent of bioavailability of theophylline, which is absorbable from the entire gastrointestinal tract.

Yuveraj singh Tanwar *et al*., 2007, developed and evaluated floating microspheres of verapamil hydrochloride for improving the drug bioavailability by prolongation of gastric residence time. The microspheres were prepared by solvent diffusion evaporation method using Cellulose acetate, Acrycoat S100 and Eudragit S100 as a rate retarding polymers. The floating microsphere of verapamil hydrochloride prepared with cellulose acetate, may provided a convenient dosage convenient dosage form for achieving best performance regarding flow, release and floating properties.

S.Sunggthongjeen *et al*., 2006, done a project on preparation and in-vitro evaluation of a multi unit floating drug delivery system based on gas formation technique. The system consist of the drug containing core pellets prepared by extrusion-spheronization process, which are coated with double layer of an inner effervescent layer (sodium bicarbonate) and an outer gas entrapped polymeric membrane of an aqueous colloidal polymer dispersion (Eudragit RL30D, RS30D) only the system using Eudragit RL30D as a gas entrapped polymeric membrane could float. Both the rapid floating and the sustained release properties were achieved in the multiple unit floating drug delivery systems developed in this study.

Yasunori Sato *et al*., 2004, done a project of In-vitro floating and drug releasing behaviours of hollow microspheres (micro balloons) prepared by the emulsion solvent diffusion method utilizing enteric acrylic polymers co-dissolved with drug in a mixture of Dichloromethane and Ethanol. In addition by incorporating a polymer such as HPMC within the shell of micro balloons the release rate of

riboflavin from the micro balloons could be controlled while maintaining high buoyancy.

Umamaheshwari *et al*., 2003, done a project on floating microspheres bearing acetohydroxamic acid for the treatment of Helicobacter pylori. Floating polycarbonate microsphere, which have the ability to reside in the gastro intestinal tract for an extended period, were prepared by emulsion solvent evaporation technique. In-vitro studies confirmed the excellent floating properties of polycarbonate microspheres.

Kamel A.H.EL *et al*., 2001, prepared and evaluated ketoprofen floating oral delivery system. The floating microparticle prepared by emulsion solvent diffusion technique. Four different ratios of Eudragit S100 with Eudragit RL were used to form the floating microparticles. The formulation containing ES: ERL 1:1 exhibited high percentage of floating particles in all examined media.

CHAPTER-IV

AIM OF THE WORK

Oral drug administration still remains the favoured route of choice for delivery of drugs into systemic circulation. Some drugs have perfect characteristics for good absorption throughout the g.i.t while the others present difficulties due to narrow absorption window in stomach and proximal gut, stability problems in intestinal fluids, poor solubility in intestine or requirement of local action in the stomach. Rapid and unpredictable gastro intestinal transit could result in partial drug absorption from the dosage form leading to reduced efficacy of the administered dose.

Perindopril erbumine {2-Methylpropan-2-amine (2*S*, 3aS, 7aS)-1-[(2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl) butyl] amino] propanoyl]octahydro - 1 *H*-indole-2-carboxylate}, a newer ACE inhibitor is used in the treatment of stable coronary artery disease and hypertension. Since the perindopril erbumine is preferentially absorbed in the proximal small intestine (narrow absorption window), the drug exhibits oral bioavailability problems in conventional dosage forms.

An smart and easy way to improve drug absorption and for releasing the drug in a controlled manner is to retain a DDS above the absorption window. Because most absorption windows are thought to be located in proximal small intestine, the noticeable strategy is to retain the formulation in the stomach (i.e., gastro retention). Gastro retention can be achieved via intra-gastric floating systems, sedimentation or high density systems, swelling or expandable systems, geometry or modified shaped systems and super porous hydro gels.

High density systems have a technical difficulty in formulating a dosage form having a density of 2.4-2.8 kg/cm^2, bio-adhesive systems may be dislodged from its site of adhesion; expandable systems may expand in oesophagus or intestines or failed to reduce in size after drug absorption to permit its transit through intestine for excretion.

The attractive principle of **floating drug delivery system** is exploited by the use of **polymers** such as semi-synthetic derivatives of cellulose along with polysaccharides which float in gastric fluids with a bulk density less than 1. It remains buoyant and floats on g.i fluids prolonging GRT.

Multiple-unit floating polymeric drug delivery systems such as **floating microspheres** offer advantages of retaining the dosage form in the upper part of GIT for prolonged period and thereby releasing the drug in a controlled manner. Such floating devices show more reproducible release profiles over all-or-nothing emptying nature or dose-dumping phenomenon associated with single-unit system.

The present investigation aims to develop floating microspheres of perindopril erbumine by **double emulsification solvent diffusion method** using polymer blends of Ethyl cellulose with HPMC, Ethyl cellulose with PVP K30, Ethyl cellulose with Eudragit S100, Ethyl cellulose with PVP K90 and Ethyl cellulose alone.

CHAPTER-V

PLAN OF WORK

Step-I

PREFORMULATION STUDIES:

a) Determination of λ_{max} of perindopril erbumine in 0.1M Hydrochloric acid.

b) Calibration curve for the perindopril erbumine at λ_{max} in 0.1 M Hydrochloric acid.

c) Compatibility Studies:

   i. *Differential scanning calorimetric (DSC) studies*

   ii. *Fourier transforms infra-red spectroscopic (FT-IR) studies*

Step-II

PREPARATION OF FLOATING MICROSPHERES:

Preparation of floating microspheres of perindopril erbumine by using different concentrations of mixed polymers (EC, EC+HPMC, EC+PVP K30, EC+ES100, EC+PVP K 90) by using double emulsion solvent diffusion technique.

Step-III

CHARACTERIZATION OF FLOATING MICROSPHERES:

a) Determination of percentage yield

b) Determination of particle size

c) Determination of Entrapment Efficiency

d) Determination of *in-vitro* Percentage Buoyancy

e) *In-vitro* release studies

f) Kinetic analysis of release data.

Step –IV

SELECTION OF BEST FORMULATION

The best formulation is selected depending on the results obtained from

- ✓ Percentage yield
- ✓ Entrapment efficiency
- ✓ Percentage buoyancy
- ✓ *In-vitro* release patterns
- ✓ Release kinetics

Step-V

EVALUATION OF BEST FORMULATION

1. *X-ray* diffraction studies
2. Scanning Electron Microscopic *(SEM)* analysis
3. *In-vivo* studies *X-Ray* studies.

CHAPTER-VI

MATERIALS AND EQUIPMENTS

Name of the material	Manufacturer
Perindopril Erbumine	Gift sample from Orchid Pharma chennai, India.
HPMC K4M	Gift samples from Orchid Pharma, chennai, India.
Ethyl Cellulose	Gift sample from Orchid Pharma, chennai, India.
Polyvinylpyrrolidine K30	Nice Chemicals,kochi, India.
Polyvinylpyrrolidine K90	Nice Chemicals,kochi, India.
Eudragit S100	Gift sample from Orchid Pharma, chennai, India.
Liquid paraffin	Universal Scientific Appliances, India.
Acetone	Central Drug House (P) Ltd, New Delhi, India.
Isopropyle alcohol	Central Drug House (P) Ltd, New Delhi, India.
Dichloromethane	Central Drug House (P) Ltd, New Delhi, India.
Ethanol	Central Drug House (P) Ltd, New Delhi, India.
Span 80	Universal Scientific Appliances
Hydrochloric acid	Nice Chemicals,kochi, India.
Cyclohexane	Central Drug House (P) Ltd, New Delhi, India.

EQUIPMENTS:

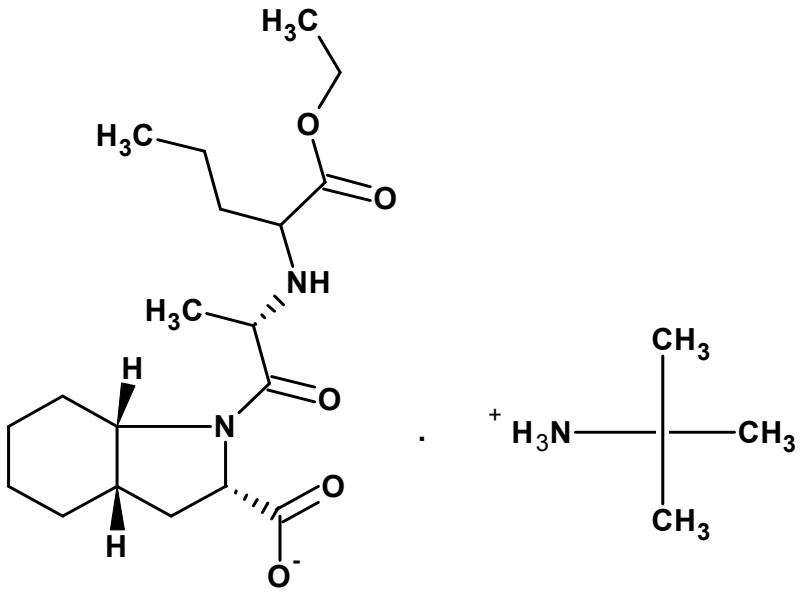
Electronic Weighing Balance	A & D Company HR 200, Japan
Mechanical Stirrer (Lab Stirrer)	Remi Motors,India
UV Visible Spectrophotometer	Shimadzhu, Japan
Digital Tablet Dissolution Test	Disso 2000, Lab India
Incubator	Tempo Industrial Corporation, Bombay,
Hot air oven	Rands Instruments, chennai, India
X-ray machine	Stallion 20, Elpro International Ltd.
Differential Scanning Calorimeter	DSC 60 Shimadzu, Japan

CHAPTER-VII

DRUG PROFILE

PERINDOPRIL ERBUMINE

Structure:



Chemical name:

(2S, 3αŚ 7αŚ)-1-[(S)-N-[(S)-1-Carboxyl- butyl] alanyl] hexahydro-2- indoline carboxylic acid, 1-ethyl ester, compound with tert-butylamine (1:1).

Empirical formula:

$C_{19}H_{32}N_2O_5C_4H_{11}N$.

Description:

Nature : white crystalline powder.

Solubility : freely soluble in water, alcohol and chloroform

Log p (Octanol/water) : 2.6

Melting point : 126-128˚C

Molecular weight : 441.61

Identification:

λ_{max} at 208 nm in UV spectrophotometer.

Pharmacodynamic Properties:

Perindopril an angiotensin –converting enzyme inhibitor, perindopril is a prodrug which is converted to active metabolite perindoprilat in liver. Perindoprilat the active metabolite competes with angiotensin converting enzyme blocking the conversion of angiotensin l to angiotensin II. It is a vasoconstrictor and a negative feedback mediator for renin activity. The Lower concentrations results in decrease in blood pressure and an increase in plasma renin. Perindoprilat may also act on kininase II; an enzyme identical to ACE that degrades vasodilator bradykinin.

Pharmacokinetic properties:

Absorption:

Rapid absorption after oral administration.

T max is 1 hour for parent compound, 3to7 hour for active metabolite.

Oral bioavailability : 75% (perindopril)

25% (perindoprilat)

Metabolism

30 -60 % perindopril is converted to active metabolite perindoprilat in liver by the enzyme.

Excretion:

Total body clearance: 219-362ml/min

Mean renal clearance: 23.3-28.6ml/min

Therapeutic indications

- Hypertension
- Stable coronary artery disease

Dose

4 mg and 8mg.maximum dose is 16 mg/day

Adverse effects

- Postural hypotension
- Hyperkalemia
- Cough
- Angio edema
- Neutropenia
- Agranulocytosis

- Anaphylactoid reactions
- Nausea
- Vomiting
- Dizziness

Drug interactions

Diuretics:

Patients on diuretics and especially those started recently, may occasionally experience an excessive reduction of blood pressure after initiation of perindopril erbumine therapy. The rate and extent of perindopril absorption and elimination are not affected by concomitant diuretics. The bio availability of perindoprilat was reduced by diuretics, however, and this was associated with a decrease in plasma ACE inhibition.

Potassium supplements and potassium-sparing diuretics:

Perindopril erbumine may be increase serum potassium because of its potential to decrease aldosterone production. Use of potassium sparing diuretics (spironolactone, amiloride, triamterene and others), potassium supplements or other drugs capable of increasing serum potassium (indomethacin, heparin, cyclosporine and others) can increase the risk of hyperkalemia. Therefore, if concomitant use of such agents is indicated, they should be given with caution and the patient's serum potassium should be monitored frequently.

Lithium:

Increased serum lithium and symptoms of lithium toxicity have been reported in patients receiving concomitant lithium and ACE inhibitor therapy. These drugs should be co administered with caution and frequent monitoring of serum lithium concentration is recommended. Use of a diuretic may further increase the risk of lithium toxicity.

Digoxin

A controlled pharmacokinetic study has shown no effect on plasma digoxin concentrations when co administered with perindopril erbumine, but an effect of digoxin on the plasma concentration of perindopril/perindoprilat has not been excluded.

Over dose & Treatment:

Symptoms associated with over dosage of ACE inhibitors may include hypotension, circulatory shock, electrolyte disturbances, renal failure, hyperventilation, tachycardia, palpitations, bradycardia, dizziness, anxiety, and cough. The recommended treatment of over dosage is intravenous infusion of normal saline solution. If hypotension occurs, the patient should be placed in the shock position, if available, treatment with angiotensin II infusion and /or intravenous catecholamine may also be considered. Perindopril may be removed from the general circulation by hemodialysis. Pacemaker therapy is indicated for therapy-resistant bradycardia. Vital signs, serum electrolytes and creatinine concentrations should be monitored continuously.

Prescription:

Yes

Generic available:

Immediate- release tablets

Preparations;

Immediate release tablets -4mg and 8mg. Maximum dose is 16mg/day.

Storage:

It should be stored in a cool, dark and dry place

Special precautions:

- Don't take potassium supplements without seeking medical advice.
- Don't take during pregnancy.

Contra-indications:

- Hypersensitivity to perindopril
- History of angio edema
- During pregnancy
- Hypotension

Brand names:

1. Aceon
2. Coversyl plus
3. Povinace
4. Apoperindopril

CHAPTER-VIII

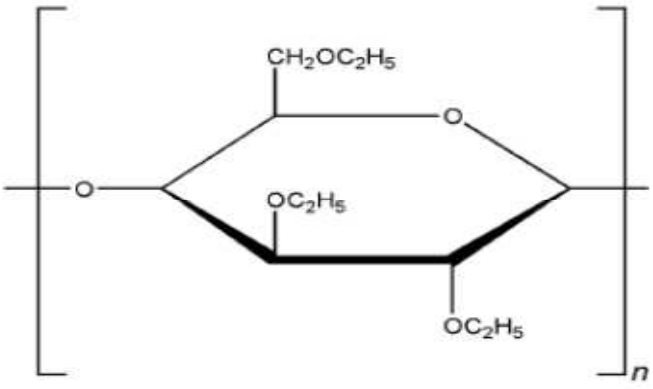
POLYMERS AND EXCIPIENTS PROFILE

ETHYL CELLULOSE

Synonyms:

- ✓ Aquacoat ECD
- ✓ Aqualon
- ✓ Ethocel
- ✓ Surelease

Structure:



Empirical formula:

- ✓ $C_{12}H_{23}O_6(C_{12}H_{22}O_5)_nC_{12}H_{23}O_5$

Molecular weight:

- ✓ 40 0000

Description:

- ✓ Color: White to light tancoloured powder.
- ✓ Odour: Odourless.
- ✓ Taste: Tasteless.

Melting point:

- ✓ 165^0 - 185^0 C

Solubility:

- ✓ Practically insoluble in propylene glycol, glycerine and water
- ✓ Freely soluble in chloroform, ethanol, ethyl acetate, methanol and toluene.

Functional Category:

- ✓ Coating agent.
- ✓ Flavouring fixative.
- ✓ Tablet binder.
- ✓ Tablet filler.
- ✓ Viscosity-increasing agent.

Storage Conditions:

- ✓ It should be stored at a temperature not exceeding 328^0 C (90^0F) in a dry area away from all sources of heat.

Handling Precautions:

- ✓ To prevent fine dust clouds of ethyl cellulose from reaching potentially explosive levels in the air.
- ✓ Its combustible
- ✓ It may be an irritant to the eyes and eye protection should be worn.

Regulatory status:

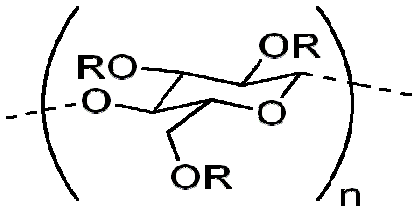
- ✓ Included in the FDA inactive ingredients **(**Raymond C *et al*., 2006**)**.

HYDROXY PROPYL METHYL CELLULOSE

Synonym:

- ✓ Hypromellose.
- ✓ Methocel

Structure:



R = H or CH_3 or $CH_2CH(OH)CH_3$

Empirical formula:

- ✓ It is partly O-methylated and O-(2-hydroxy propylated) cellulose. (Ph Eur 2005). It is available in several grades depending upon the viscosity and extent of substitution.

Molecular weight:

- ✓ 10 000 – 1 5 00 000

Description:

- ✓ **Colour:** White or creamy-white fibrous or granular powder.
- ✓ **Odour:** Odorless
- ✓ **Taste:** Tasteless

Solubility:

- ✓ Soluble in cold water, forming a viscous colloidal solution,
- ✓ Practically insoluble in chloroform, ethanol (95 %) and ether,
- ✓ Soluble in mixtures of ethanol and dichloromethane,

- ✓ Soluble in mixtures of water and alcohol.

Functional Category:

- ✓ Coating agent.
- ✓ Film- former.
- ✓ Stabilizing agent.
- ✓ Tablet binder.
- ✓ Viscosity increasing agent.

Typical Viscosity values for 2 % (w/v) aqueous solutions of different viscosity grades of HPMC at 20°C

Methocel K100 Premium LVEP	:	100
Methocel K4M Premium	:	4000
Methocel K15M Premium	:	15000
Methocel K100M Premium	:	100 000
Methocel E4M Premium	:	4000
Methocel F50 Premium	:	50
Methocel E10M Premium CR	:	10 000
Methocel E3 Premium LV	:	3
Methocel E5 Premium LV	:	5
Methocel E6 Premium LV	:	6
Methocel E15 Premium LV	:	15
Methocel E50 Premium LV	:	50
Metolose 60SH	:	50, 4000, 10 000
Metolose 65SH	:	50, 400, 1500, 4000

Metolose 90SH : 100, 400, 4000, 15 000

Storage Conditions:

- ✓ It should be stored in a well-closed container, in a cool, dry place.

Handling Precautions:

- ✓ Hypromellose dust may be irritant to the eyes and eye protection is recommended
- ✓ Excessive dust generation should be avoided to minimize the risks of explosion.
- ✓ Hypromellose is combustible **(**Raymond C *et al*., 2006**)**.

POLYMETHACRYLAT (EUDRAGIT S100)

Synonyms

- ✓ Acryl-EZE; Acryl-EZE MP; Eastacryl 30D; Eudragit;
- ✓ KollicoatMAE 30 D; Kollicoat MAE 30 DP;
- ✓ polymeric methacrylates.

Nonproprietary names

BP: Methacrylic acid–ethyl acrylate copolymer (1: 1)

PhEur:

Acidum methacrylicum et ethylis acrylas polymerisatum (1: 1)

Acidum methacrylicum et ethylis acrylas polymerisatum

(1 : 1) dispersio 30 per centum

Acidum methacrylicum et methylis methacrylas Polymerisatum (1 : 1)

Acidum methacrylicum et methylis methacrylas Polymerisatum (1 : 2)

Copolymerum methacrylatis butylati basicum

Polyacrylatis dispersion 30 per centum

USPNF:

Ammonio methacrylate copolymer

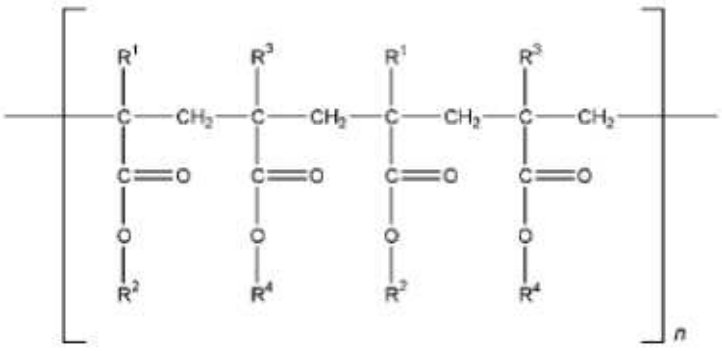
Methacrylic acid copolymer

Methacrylic acid copolymer dispersion

Chemical name **:** Poly(methacrylic acid, methyl methacrylate) 1 : 2

Empirical formula **:** $(C5\ H_8\ O_{2)\ n}$

Structural formula



$R^1, R^3, R^4 = CH3$

$R^2 = H$

Description

- ✓ Nature : White free flowing powder
- ✓ Solubility : Soluble in acetone and alcohol
- ✓ Molecular weight : ≥100 000

Functional categories

- ✓ Film former
- ✓ Tablet binder
- ✓ Tablet diluents

Properties

- ✓ Loss on drying : ≤ 5.0%
- ✓ Methyl methacrylate and methacrylic acid : ≤ 0.1%
- ✓ Sulfated ash : ≤ 0.1%
- ✓ Apparent viscosity : 50–200 mPa s
- ✓ Stablility and storage : Dry powders are stable
  - for at least 3 years if stored in a tightly closed
  - container at less than 30ºC.
- ✓ Assay : Methacrylic acid units 27.6–30.7%

Safety:

Polymethacrylate copolymers are widely used as film-coating materials in oral pharmaceutical formulations. They are also used in topical formulations and are

generally regarded as nontoxic and nonirritant materials. A daily intake of 2 mg/kg body-weight of Eudragit (equivalent to approximately 150mg for an average adult) may be regarded as essentially safe in humans **(**Raymond C *et al.,* 2006**)**.

Handling Precautions:

- ✓ Observe normal precautions appropriate to the circumstances and quantity of material handled. Additional measures should be taken when handling organic solutions of polymethacrylates.
- ✓ Eye protection, gloves, and a dust mask or respirator are recommended. Polymethacrylates should be handled in wellventilated environment and measures should be taken to prevent dust formation.
- ✓ Acute and chronic adverse effects have been observed in workers handling the related substances methyl methacrylate and poly(methyl methacrylate) (PMMA).(19,20) In the UK, the occupational exposure limit for methyl methacrylate has been set at 208 mg/m3 (50 ppm) long-term (8-hour TWA), and 416 mg/m3 (100 ppm) short-term.

POLYVINYLPYRROLIDONE

Synonyms

- ✓ E1201; Kollidon; Plasdone; poly[1-(2-oxo-1-pyrrolidinyl)ethylene];
- ✓ polyvidone; polyvinylpyrrolidone; PVP; 1-vinyl-2-pyrrolidinone Polymer.

Chemical Name

1-Ethenyl-2-pyrrolidinone homopolymer

Empirical Formula and Molecular Weight

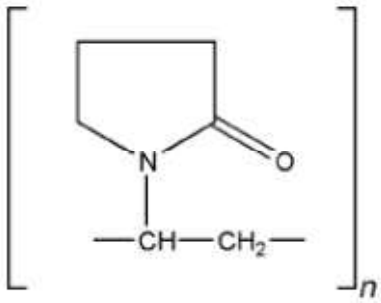
(C6H9NO)n 2500–3 000 000

The USP 28 describes polyvinylpyrrolidone as a synthetic polymer consisting essentially of linear 1-vinyl-2-pyrrolidinone groups, the differing degree of polymerization of which results in polymers of various molecular weights **(**Raymond C *et al*., 2006**)**. It is characterized by its viscosity in aqueous solution, relative to that of water, expressed as a K-value, in the range 10–120. The K-value is calculated using Fikentscher's equation.

Approximate molecular weights for different polyvinylpyrrolidone grades are shown in given Table (Raymond C *et al*., 2006**)**

K value	Molecular weight
12	2500
15	8000
17	10000
25	30000
30	50 000
60	400000
90	10 00000
120	30 00000

Structural Formula:



Functional Category:

Disintegrant; dissolution aid; suspending agent; tablet binder.

Applications in Pharmaceutical Formulation:

- ✓ Although Polyvinylpyrrolidone is used in a variety of pharmaceutical formulations **(**Raymond C *et al*., 2006**)**, it is primarily used in solid-dosage forms. In tableting, polyvinylpyrrolidone solutions are used as binders in wet granulationprocesses.

- ✓ Polyvinylpyrrolidone is also added to powder blends in the dry form and granulated in situ by the addition of water, alcohol, or hydroalcoholic solutions. Polyvinylpyrrolidone is used as a solubilizer in oral and parenteral formulations and has been shown to enhance dissolution of poorly soluble drugs from solid-dosage forms. Polyvinylpyrrolidone solutions may also be used as coating agents.

- ✓ Polyvinylpyrrolidone is additionally used as a suspending, stabilizing, or viscosity increasing agent in a number of topical and oral suspensions and solutions.

- ✓ The solubility of a number of poorly soluble active drugs may be increased by mixing with Polyvinylpyrrolidone.

Uses of Polyvinylpyrrolidone.

- ✓ Carrier for drugs
- ✓ Dispersing agent

- ✓ Eye drops
- ✓ Suspending agent
- ✓ Tablet binder, tablet diluent, or coating agent

Description

- ✓ Polyvinylpyrrolidone occurs as a fine, white to creamy-white colored, odorless or almost odourless, hygroscopic powder.
- ✓ Polyvinylpyrrolidone with K-values equal to or lower than 30 are manufactured by spray-drying and occur as spheres.
- ✓ Polyvinylpyrrolidone K-90 and higher K-value Polyvinylpyrrolidone are manufactured by drum drying and occur as plates.

Solubility:

Freely soluble in acids, chloroform, ethanol (95%), ketones, methanol, and water; practically insoluble in ether, hydrocarbons, and mineral oil. In water, the concentration of a solution is limited only by the viscosity of the resulting solution.

Stability and Storage Conditions

Polyvinylpyrrolidone may be stored under ordinary conditions without undergoing decomposition or degradation. However, since the powder is hygroscopic, it should be stored in an airtight container in a cool, dry place.

Incompatibilities

- ✓ Polyvinylpyrrolidone is compatible in solution with a wide range of inorganic salts, natural and synthetic resins, and other chemicals.

- ✓ It forms molecular adducts in solution with sulfathiazole, sodium salicylate, salicylic acid, phenobarbital, tannin, and other compounds.

Safety

Polyvinylpyrrolidone is widely used as an excipient, particularly in oral tablets and solutions **(**Raymond C *et al*., 2006**)**. When consumed orally, Polyvinylpyrrolidone may be regarded as essentially nontoxic since it is not absorbed from the gastrointestinal tract or mucous membranes.

Polyvinylpyrrolidone additionally has no irritant effect on the skin and causes no sensitization.

Handling Precautions

Observe normal precautions appropriate to the circumstances and quantity of material handled. Eye protection, gloves, and a dust mask are recommended.

CHAPTER-IX

EXPERIMENTAL DETAILS

I. CALIBRATION OF PERINDOPRIL ERBUMINE:

a) Preparation of dissolution medium:

***0.1M Hydrochloric Acid*:**

Dilute 8.5 ml of hydrochloric acid in distilled water and the volume is made up to 1litre

b) Calibration Curve of Perindopril Erbumine:

To the powder containing 10mg of Perindopril Erbumine, 10ml of distilled water is added and the volume is made up to 100ml with 0.1M hydrochloric acid. Dilutions are made to get the concentration of 5 to 50µg/ml. 10µg/ml solution is scanned in (UV) Spectrophotometer to find out the λ_{max} and absorbance is measured at the obtained λ_{max} (208nm). The calibration graph is plotted by taking the concentration on X axis and respective absorbance in Y axis, to get a straight line as per like Beers law. The regression value is determined.

II. PREFORMULATION (COMPATABILITY) STUDIES:

a) Differential Scanning Colorimetric studies (DSC):

DSC is performed using Q200 V24.4 thermal analyzer. The instrument is calibrated with indium standard. Accurately weighed (it varies from 3mg -25mg) samples are placed in an open type ceramic sample pans. Thermo grams are obtained by heating the

sample at a constant heating rate of 8˚C/minute. A dry purge of argon gas (60ml/min) is used for all runs. Samples are heated from 37˚-400˚C.

b) Fourier Transform-Infra Red (FT-IR) Studies:

The possibility of drug–excipients interactions are further investigated by FT-IR. The FT-IR graph of pure drug and combination of drug with excipients are recorded. The analysis is performed by using (shimadzu FT-IR, Japan) spectrometer. The scanning range is 450-4000 cm^{-1} and the resolution is 4 cm^{-1} sample is prepared in KBr pellets.

III. PREPARATION OF FLOATING MICROSPHERS:

Floating microspheres are prepared by using water-in-oil-in-oil (w/o/o) double emulsion solvent diffusion method (K. Rama Rao *et al*., 2005, Bipul Nath *et al*., 2010) using different rations of polymers to drug. The polymers is composed of Ethyl cellulose (F1-F4), Ethyl cellulose with Hydroxyl Propyl Methyl Cellulose (F5-F8), ethyl cellulose With Polyvinyl Pyrrolidine K30 (F9-F12), ethyl cellulose with Eudragit S 100 (F13-F16), and ethyl cellulose with Polyvinyl Pyrrolidine K90 (F17-F20). Briefly drug and polymer mixture are dissolved in the mixed solvent system consisting of Acetone and Dichloromethane in a1:1 ratio for F1 to F8, Acetone, Ethanol and Dichloromethane in a 1:1:1 ration for F9 to F12 and F17 to F20, Ethanol, Isopropyl alcohol and Dichloromethane (Yuveraj Singh *et al*.,2007) in a 1:1:1 ratio for F13 to F16.

The initial w/o emulsion is prepared by adding 4ml of water to the drug-polymer solution while stirring using a mechanical stirrer (lab stirrer, Remi motors, India) at 500 rpm for 5 min. This w/o primary emulsion is slowly added to 200ml (V.S Mastiholimath *et al*., 2008) of light liquid paraffin, the second a oil phase containing 0.1% span80 as a

surfactant while stirring at 1000 rpm. After 2 h, 10ml of cyclohexane (non solvent) is added to harden the microspheres and the stirring is continued for a further 1 h and the hardened microspheres are collected by filtration and washed with three portions of 50ml of cyclohexane and air dried for 12 h. Table (2) shows the composition of various prepared floating microspheres formulations.

IV. CHARACTERIZATION OF FLOATING MICROSPHERES

a) Percentage Yield:

The yield percentage of the produced microspheres is (A.H.EL-Kamel *et al*., 2001; Pratim.K Choudhury & Mousumi Kar 2009) calculated for each batch by dividing the whole weight of product (M) by the total expected weight of drug and polymer (Mo)

$$\text{Percentage yield} = \frac{\text{Weight of microspheres (M)}}{\text{Total expected weight of drug and polymer (Mo)}} \times 100$$

b) Particles Size Analysis

Particle size distribution is analyzed by (V.S Mastiholimath *et al*., 2008) placing of the formulated microspheres in a set of standard test sieves and shaking it for a particular time interval. The particles collected in each sieve were weighed and the percentage particles retained on each sieve was calculated. The average diameter of the microspheres is represented by the geometric mean diameter obtained.

c) Drug Entrapment Efficiency:

The amount of perindopril erbumine present in the microspheres is determined by extracting into acid buffer (0.1M hydrochloric acid). Microspheres (Pratim.K Choudhury & Mousumi Kar 2009) are crushed and powdered by using a mortar and pestle and accurately weighed amount of this powder is extracted into 100ml of acid buffer (0.1M hydrochloric acid) by stirring at 1000 rpm for 2 h. The solution is filtered; suitable dilutions are made and estimated for perindopril erbumine content spectrophotometrically at 208 nm.

Theoretical drug loading in microspheres is estimated by using (M.saravanan, B. Anupama2011; Pratim.K Choudhury & Mousumi Kar 2009) the following formula

$$\textit{Theoretical drug loading}\ (\%) = \frac{\text{Weight of drug}}{\text{Weight of microspheres}} \times 100$$

The Entrapment efficiency of prepared microspheres is calculated by using

(Malaykumar *et al*., 2006; Chudiwal P.D *et al*., 2009; Pandey Manisha *et al*., 2010) the following formula

$$\textit{Entrapment efficiency}\ (\%) = \frac{\text{Experimental drug content}}{\text{Theoretical drug content}} \times 100$$

d) In-Vitro Percentage Buoyancy Studies:

The floating microspheres (300mg) are spread over the surface of the dissolution medium (simulated gastric fluid pH 1.2) containing Tween 20 (0.02% w/v) that

is agitated by a paddle rotated at 100 rpm (R.B.Uma maheswari *et al*., 2003; V.S Mastiholimath *et al*., 2008). After agitation for a predetermined time interval, the microspheres that floated over the surface of the medium and those settled at the bottom of the jar are recovered separately. After drying, each fraction of the microspheres is weighed and the buoyancy of the microspheres if calculated by the following equation:

$$\textit{Percentage Buoyancy} = Qf / (Qf + Qs)$$

Where Qf and Qs are the weight of the floating and the settled microspheres, respectively.

e) *In-vitro* Release Studies:

In vitro release studies are performed in USP type II paddle (V.S.Mastiholimath *et al*., 2008; Ghodake J.D.*et al*., 2010; S.Sungthonjeen *et al*., 2006) apparatus for 12 hours. The microspheres are placed in the dissolution medium of 900ml 0.1M hydrochloric acid in the dissolution apparatus. The paddle is rotated at 100 rpm maintained at 37˚C. 5ml samples are withdrawn every 15 min for the first hour and every 30 min up to 11 hours. Sink conditions are maintained after each sampling. Samples are analyzed at 208 nm using UV spectrophotometer. The studies are done in triplicate.

f) Kinetic Analysis:

The *in-vitro* release profiles obtained from the floating microspheres are (Paulo Costa *et al.*,2001) fitted to zero order, first order, Hixson Crowell, Korsemeyer & peppas model kinetics, to find out the mechanism of drug release.

Zero order	:	Qt = Qo + Ko.t
First order	:	In Qt = In Qo + Ko.t
Hixson-Crowell	:	Qo1/3 – Qt1/3 + K.t
Higuchi	:	Q = KH.t1/2
Korsmeyer- peppas	:	Mt/Mo = a.tn

Fitness of release profiles to linear equations is assessed by comparing the coefficients of determination (r) values.

For cylinder type of systems (Paulo Costa et al., 2001),

n<0.5 : classical Fickian diffusion

n=0.5to1.0 : anomalous Non Fickian transport i.e. coupled drug diffusion in the hydrated matrix and polymer relaxation (Indicators of both phenomenons)

n=1.0 : case II relaxational release transport – zero order release (Polymer relaxation or swelling controlled systems)

n > 1.0 : super caseII transport.

V. SELECTION OF BEST FORMULATION

The best formulation is selected depending on the results obtained from

- ✓ Percentage yield
- ✓ Entrapment Efficiency
- ✓ Percentage buoyancy

- ✓ *In-vitro* Release Studies
- ✓ Release kinetics.

a) EVALUATION OF BEST FORMULATION

The selected best formulation is subjected to,

1. *X-Ray diffraction studies*
2. *Scanning Electron Microscopy (SEM)* analysis
3. *In-vivo X-Ray* studies

1) *X-Ray Diffraction* Studies:

Perindopril erbumine, perindopril erbumine loaded Ethyl cellulose/HPMC microspheres , physical mixture of perindopril erbumine and ethyl cellulose/HPMC are subjected to X-ray diffraction study in an X-ray diffractometer (XD, Shimadzu, Japan) within the range 10-80˚ of 2θ (Yuanfen Liu et al., 2011).

2) *Scanning Electron Microscopy* (SEM):

Scanning electron microscopy (SEM) studies are performed (Garg and Gupta, 2010) to determine the porous/ hollow nature of the microspheres. Surface morphology of microspheres is also noted.

3) *In-Vivo X- Ray* Studies:

The *in-vivo* studies approved by institutional animal ethical committee reference No.14024/E1/4/2011 and are performed on healthy male albino rabbit weighing 2-3 kg. The animal is fasted overnight but allowed to take water libitum. Then 60ml of 5%

dextrose solution is given immediately before administering the microspheres by using stomach tube (NO.12French catheter) and 50ml syringes.

The microspheres are made opaque by incorporating Barium sulphate (BaSO4) instead of drug. The rabbit is exposed to X-ray imaging in the abdominal region, and photographs are taken at 0, 4, 8, 12 hrs after administration of microspheres. At hourly intervals 60 ml of 5% dextrose solution is given to maintain optimum fluid level in the stomach. The gastric residence time is observed.

CHAPTER-X

RESULTS AND DISCUSSION

I. CALIBRATION OF PERINDOPRIL ERBUMINE:

The λ_{max} of perindopril erbumine was determined by scanning the 10 μg/ml solution of the drug using UV spectrophotometer and was found to be **208**nm is shown in Fig:14 (European Pharmacopoeia 2009).The absorbance of the solutions (5 – 50 µg/ml) was measured in UV spectrophotometer at 208 nm. The correlation coefficient was found to be γ = **0.99985**. The results were given in Table: 1 and the calibration graph of perindopril erbumine were shown in Fig: 15

II. PREFORMULATION (COMPATABILITY) STUDIES:

a) Differential Scanning Colorimetric Studies (DSC):

The DSC thermo grams of pure drug and the different polymers were shown in the Fig.16 an endothermic peak corresponding to the melting point of pure drug was important in all the drug polymer mixture, which suggested clearly that there was **no interaction** between the drug and the polymers and the drug was existed in its unchanged form.

b) Fourier Transform-Infra Red(FT-IR) Studies:

FT-IR spectrum of the drug and polymers are shown in the fig.17 The spectrum of the drug had characteristic peaks of C-H stretching (VF 2929, 2848, 2750), C=O stretching (VF 2640&1739 cm^{-1}), **hydrogen** bonded acids (VF 2551.61 cm^{-1}), C-H bending (VF 1392 cm^{-1}), OH bending (VF 1315, 1292, 1205 cm^{-1}), aromatic rings (VF 1566 cm^{-1}), C-H rocking (939, 750, 703, 475 cm^{-1}) thus indicating the identity and purity of the drug.

All those characteristic peaks were also found in the spectrum of drug and polymer combinations and there was no change in the existing peaks. This clearly indicated that there was **no interaction** between the drug and the polymer and the drug was present in its unchanged form.

III. PREPARATION OF FLOATING MICROSPHERES:

Floating microspheres were prepared by using **water-in-oil-in-oil(w/o/o) double emulsion solvent diffusion** method(K.Rama Rao *et al*.,2005, Bipul Nath *et al*.,2010), using different ratio of mixed polymers to perindopril erbumine.Table (2) shows the composition of various prepared floating microspheres formulations. The perindopril erbumine and polymer (Ethyl cellulose) was in the ratio of 1:1, 1:2, 1:3 and 1:4 for F1, F2, F3 and F4 respectively,Perindopril Erbumine and mixed polymer(Ethyl cellulose with HPMC) in the ratio of 1:1, 1:2, 1:3 and 1:4 for F5, F6, F7 and F8 respectively,Perindopril Erbumine and mixed polymer(Ethyl cellulose with PVP K30) in the ratio of 1:1, 1:2, 1:3 and 1:4 for F9, F10, F11 and F12 respectively,Perindopril Erbumine and mixed polymer(Ethyl cellulose with Eudragit S100)in the ratio of 1:1, 1:2, 1:3 and 1:4 for F13, F14, F15 and F16 respectively andPerindopril Erbumine and mixed polymer(Ethyl cellulose with PVP K90) in the ratio of 1:1, 1:2, 1:3 and 1:4 for F17, F18, F19 and F20 respectively.

The preparation of microsphere was carried out by emulsifying an aqueous solution in to solution of drug and polymers in mixed solvent system comprising of Acetone and dichloromethane in equal volume for F1 to F8, Acetone ,Ethanol and Dichloromethane in equal volume for F9 to F12 and F17 to F20, Ethanol, Isopropyl alcohol and Dichloromethane in equal volume for F13 to F16, followed by

emulsification of this primary emulsion (w/o) in to an external oil phase (liquid paraffin) to form a water in oil in oil (w/o/o) emulsion.

Microspheres were formed after a series of steps like solvent extraction, solvent evaporation and addition of a non-solvent.The solvents of the system were removed by a combination of extraction and evaporation. It is very important to carefully select the solvent combination and processing medium to enable the formation of double emulsion and solvent extraction and evaporation by a combination.

Acetone is a unique organic solvent which is polar, water miscible and oil immiscible and dichloromethane is non polar and oil miscible (Badri viswanathan*et al*. 1999) so, during the formation of microspheres dichloromethane was extracted by liquid paraffin and acetone was evaporated during stirring.

Each step of microsphere preparation was intensely observed to understand the effect of particle size, total entrapment and release profile of the drug loaded microspheres. After introduction of w/o primary emulsion in to liquid paraffin, the emulsion was stirred for 2 hr using mechanical stirrer (Remi lab stirrer), during this phase it is assumed that the droplet sizes were allowed to stabilize while some amount of dichloromethane and acetone escaped, making the emulsion droplets become more viscous.

The cyclohexane, non solvent for the polymer added at this stage might have caused the quick precipitationof the polymer leaving the surface of microspheres porous in nature.No surfactant was used for stabilizing w/o primary emulsion because ethyl cellulose itself acts as a surfactant(Melzer *et al*.,2003). Span 80 (sorbitan monooleate) was used to stabilise the secondary emulsification process.

IV. CHARACTERIZATION OF FLOATING MICROSPHERES

a) Percentage Yield:

The percentage yield of produced floating microspheres was shown in Table: 4. Increasing the polymer concentration lead to subsequent increase in its hydrophobicityconsequently, it will react better with non solvent phase (liquid paraffin) leading to more efficient precipitation of the polymer at the droplet interface with subsequent higher yield.

Increasing polymer ratio in the formulation led to increase the product yield (S.Sehra&A.S.Dhake., 2005)**.**The low percent yield in some formulations may also due to microspheres lost during successive decantation during washing process.

b) Particle Size Analysis

Formulation F4(1:4), F8(1:4), F12(1:4), F16(1:4) and F20(1:4) showed relatively larger particle size and formulation F1(1:1), F5(1:1), F9(1:1), F13(1:1) and F17(1:1) showed relatively small particle size of floating microspheres.

The polymer to drug ratio appears to influence the particle size distribution of floating microspheres (K.Rama Rao *et al*., 2005), as shown in Tables: 4.

When the polymer to drug ratio was increased, the proportion of larger particles was high, because the viscosity of the primary emulsion was increased with increase of polymer to drug ratio. Due to this increased viscosity, large emulsion droplets were formed and it was difficult to break them and, hence, they were precipitated as such leading to an increase in the mean particle size of floating microspheres, as shown in Table: 4.

c) Drug Entrapment Efficiency:

Drug entrapment efficiency was found to be 84.10%, 88.29%, 91.58%,95.97%, 85.98%, 88.29%, 93.45%, 95.79%, 66.35%, 77.25%, 79.43%, 93.45%, 67.28%, 68.69%, 78.50%, 86.44%, 71.02%, 78.51%, 91.56% and95.75% for formulation F1(1:1), F2(1:2), F3(1:3), F4(1:4), F5(1:1), F6(1:2), F7(1:3), F8(1:4), F9(1:1), F10(1:2), F11(1:3), F12(1:4), F13(1:1), F14(1:2), F15(1:3), F16(1:4), F17(1:1), F18(1:2), F19(1:3)andF20(1:4)respectively.

Among the different drug polymer ratios investigated 1:4 (F4, F8, F12, F16, and F20) drug-polymer ratio had the maximum capacity for drug entrapment as shown in Figure: 18.

Drug entrapment efficiency was increased with increasing polymer concentration in floating microspheres (Pratim.K Choudhury & Mousumi Kar 2009, Pandey Manisha *et al*., 2010,) as shown in Tables: 4.

Reason for High Entrapment Efficiency:

As the high molecular weight of the polymer (Ethyl cellulose) increased its hydrophobicity increased, leading to better precipitation of the polymer at the boundary phase of the droplets (M.S.Uddin*et al*.,2001). Consequently, partitioning of drug to the continuous phase (liquid paraffin) will be minimal.

Thehigher entrapment of the perindopril erbumine to the polymer blend (Ethyl cellulose with HPMC) may be attributed to faster precipitation of polymer at sphere interface at this drug: polymer (1:4) ratio, consequently, higher amount of drug was entrapped.

d) In-vitro Percentage Buoyancy Studies:

The buoyancy percentage for all batches was almost above 60% (Fig 19), which was studied for 12hrs, in dissolution medium (simulated gastric fluid pH 1.2) containing Tween 20 (0.02% w/v) without enzymes. The average buoyancy in percentage was found to be **62.06% to 82.77%**. The highest percentage was obtained with formulation F4 (1:4), F8 (1:4), F12 (1:4), F16 (1:4) and F20 (1:4). In general with increase in the amount of polymer blend (Ethyl cellulose + HPMC), there was an increased in buoyancy percentage (Pandey Manisha *et al*., 2010). From the Table: 4 it was concluded that on increasing polymer concentration simultaneously percentage buoyancy also increased.

e) In-Vitro Release Studies:

The cumulative percentage drug release after 12 hr was found to be 80.83%,74.70%, 65.98% and 66.63% for the formulations of F1 to F4, 82.24%, 76.26%, 74.56% and 68.13% for the formulations of F5 to F8, 77.78%, 72.65%, 71.68% and 69.49% for the formulations of F9 to F12, 82.11%, 73.30%, 72.86% and 70.12% for the formulation of F13 to F16 and 86.95%, 78.05%, 73.36% and 66.84% for the formulation of F17 to F20. It was found that the drug release was prolonged up to 12 hrs.The results are shown in figure 21, 21, 22, 23, & 24. It was also observed that as the polymer ratio increased the drug release was decreased (V.S.Mastiholimath *et al*., 2008).

The *in-vitro* release of perindopril erbumine from floating microsphere was biphasic with the initial burst effect which was varied from 15% to 27% depending on the polymer-to-drug ratio (K.Rama Rao *et al*., 2005). The initial burst effect was due to presence of drug particle on the surface of the microspheres, which was exposed by scanning electron microscope studies.

The initial burst effect may also attribute as a desired effect to ensure initial high plasma concentration of drug to produce pharmacological action. In order to keep the total surface area of the microsphere constant. The effect of retardation on the release rate depends on the polymer to drug ratio. As the concentration of polymer increased with respect to drug which may be attributed to the slower rate of diffusion of dissolution medium into the microspheres due to increased density of the polymer matrix at higher concentration result in an increased diffusional path length. This may decrease the overall drug release from the polymer matrix.

After the total drug release (12hr) the floating microspheres were collected and observed under SEM for the surface change occurred after dissolution. Furthermore, smaller floating microspheres were formed at a lower polymer concentration and have a longer surface area exposed to dissolution medium, giving rise to faster drug release.

***f) Kinetic Analysis*:**

The *in-vitro* release profile was applied on various kinetic models in order to find out mechanism of drug release. The correlation coefficient of F1 to F20 formulations for zero order, Higuchi, Hixon-crowell and first order equations was shown in Table: 5. Formulation F8 was found high correlation to zero order kinetics (0.987, Figure: 25) as well as Higuchi plot (0.997, Figure: 26) rather than Hixson-crowell models.

The drug release was proportional to square root of time, indicating that the drug release from polymeric (EC+HPMC) microspheres was diffusion controlled. The data obtained was also put in Korsemeyer-peppas equation in order to find out n value (0.506 to 0.726 for F1 to F20**),** which describes the drug release mechanism by Non Fickian diffusion.

V. SELECTION OF BEST FORMULATION:

From the above results of characterization **F8** was selected as the best formulation because,

- ✓ Percentage yield :76.84%
- ✓ Entrapment efficiency :95.79%
- ✓ Percentage buoyancy :82.77%
- ✓ Cumulative % drug release :68.13%
- ✓ Release kinetics :closest linearity to zero order kinetics, Higuchi model and Non-Fickian diffusion mechanism

a) EVALUATION OF BEST FORMULATION

The selected best formulation **F8** was subjected to,

1. *X-Ray diffraction studies*
2. *Scanning Electron Microscopy (SEM)* analysis
3. *In-vivoX-Ray*studies

1) *X-Ray Diffraction* Studies:

In order to determine the physical state of the drug whether amorphous or crystalline before and after floating microspheres formulation, X-ray examination were conducted for the pure drug, the polymer mixture and the formulations of floating microspheres (Figure: 28).From *X-ray* patterns it was obvious that the pure drug exhibited crystalline characteristics peaks, polymers exhibited amorphous pattern while formulations showed

reduced crystallinity peaks of pure drug.

The amorphous state of spheres releases the drug less rapidly than the crystalline spheres (S.Freiberg and X.X.Zhu., 2004).Therefore, the lack of polymer crystallinity suggests betterdrug dispersion and increased drug polymer interactions. The drug release rate can be tailored by manipulating the degree of crystallinity; reduced crystallinity is favourable when slow release is desired.

2) Scanning Electron Microscopy (SEM):

Scanning electron microscopy(SEM) exposed the distinct, spherical shaped spheres with rough surface and presence of holes /hollow cavity due to the collapse of the wall of the microspheres during in situ drying process (Gangadharappa H. V *et al*., 2011). Thus the rate of solvent removal from the embryonic microspheres exerts an influence on the morphology of the end product.

Porous structure was observed on the surface of microspheres shell due to the rapid diffusion of the solvent, there is a possibility of rupture of some microspheres. Microspheres floated more than 12 h because of presence of pores. SEM photographs were shown in Figure: 29 and 30.

3) *In-Vivo X-Ray* Studies:

The in vivo floating behaviour of microspheres loaded with barium sulphate was investigated by radiographic images (X- ray photographs) of rabbit's stomach at specific periods.

The amount of X-ray opaque material in these microspheres was sufficient to ensure visibility by X-ray but at same time the amount of barium sulphate (100 mg) was low enough to enable the microspheres to float(Gangadharappa H. V *et al*., 2011).

The floatingmicrospheres did not adhere to the gastric mucous and floating on the gastric fluid for about more the 12 h. This was evident by the X-ray photographs taken at 0 hr, 4^{th} hr, 8^{th} hr& 12^{th} hr. It was shown in Figure: 31.

Table 1: Calibration of Perindopril Erbumine

Medium : 0.1M HCL
λ max : 208 nm

S. No.	CONCENTRATION (µg/ml)	ABSORBANCE *	STANDARD DEVIATION * (± S.D)
1.	5	0.108	0.005
2.	10	0.230	0.017
3.	15	0.338	0.009
4.	20	0.457	0.018
5.	25	0.564	0.012
6.	30	0.684	0.017
7.	35	0.784	0.023
8.	40	0.903	0.009
9.	45	1.002	0.028
10.	50	1.119	0.022
		R^2 Value = 0.99985	

* Average of three trial

Table 2: Formulation Table for Floating Microspheres of Perindopril Erbumine

Formulation code	Drug: Polymer	Liquid Paraffin (ml)	Drug (gm)	Ethyl cellulose (gm)	HPMC (gm)	PVP K30 (gm)	Eudragit S100 (gm)	PVP K90 (gm)
F1	1:1	200	0.5	0.500	-	-	-	-
F2	1:2	200	0.5	1.000	-	-	-	-
F3	1:3	200	0.5	1.500	-	-	-	-
F4	1:4	200	0.5	2.000	-	-	-	-
F5	1:1	200	0.5	0.250	0.250	-	-	-
F6	1:2	200	0.5	0.750	0.250	-	-	-
F7	1:3	200	0.5	1.250	0.250	-	-	-
F8	1:4	200	0.5	1.500	0.500	-	-	-
F9	1:1	200	0.5	0.250	-	0.250	-	-
F10	1:2	200	0.5	0.750	-	0.250	-	-
F11	1:3	200	0.5	1.250	-	0.250	-	-
F12	1:4	200	0.5	1.500	-	0.500	-	-
F13	1:1	200	0.5	0.250	-	-	0.250	-
F14	1:2	200	0.5	0.750	-	-	0.250	-
F15	1:3	200	0.5	1.250	-	-	0.250	-
F16	1:4	200	0.5	1.500	-	-	0.500	-
F17	1:1	200	0.5	0.250	-	-	-	0.250
F18	1:2	200	0.5	0.750	-	-	-	0.250
F19	1:3	200	0.5	1.250	-	-	-	0.250
F20	1:4	200	0.5	1.500	-	-	-	0.500

Table - 3

In-Vitro Release Profile of Perindopril Erbumine Floating Microspheres

Time (Hours)	Cumulative % Drug Release					
	Formulation Code					
	F1 (D:P 1:1)		F2 (D:P 1:2)		F3 (D:P 1:3)	
	Mean	±SD	Mean	±SD	Mean	±SD
0.25	12.393	1.883	11.540	2.797	6.487	1.063
0.5	16.896	1.157	18.433	0.825	11.284	0.787
0.75	21.656	0.274	21.000	0.749	13.096	0.510
1	25.943	1.258	24.110	0.545	15.790	0.515
1.5	28.926	0.482	26.966	0.350	17.799	0.269
2	32.126	1.603	29.730	0.465	20.353	0.313
2.5	34.833	0.249	32.073	0.550	21.922	0.507
3	38.536	2.255	35.416	0.600	23.505	0.271
3.5	39.796	0.778	38.270	0.574	25.441	0.517
4	41.846	1.321	40.516	0.591	26.233	0.502
4.5	43.533	2.525	43.013	0.748	28.493	1.010
5	47.503	0.814	45.646	0.531	30.851	0.515
5.5	50.707	0.754	47.083	0.510	33.044	1.20
6	53.426	0.440	46.946	1.610	35.370	0.832
6.5	56.560	0.618	51.280	1.589	39.256	0.541
7	57.546	1.051	54.560	1.309	41.868	0.543
7.5	59.146	0.507	56.723	3.136	43.980	0.786
8	62.450	3.495	58.646	2.514	44.611	1.665
8.5	67.843	1.583	61.746	1.737	46.894	1.367
9	70.456	1.650	63.733	1.753	48.897	0.840
9.5	71.873	0.649	66.723	1.046	50.393	1.219
10	73.990	1.463	68.543	1.729	54.287	0.950
10.5	76.336	1.033	71.716	0.918	56.020	0.967
11	78.276	1.516	71.983	0.666	58.255	0.642
11.5	80.473	1.153	73.400	1.140	60.657	0.885
12	80.830	0.921	74.700	0.459	65.968	0.439

Table – 3a

In Vitro Release Profile of Perindopril Erbumine Floating Microspheres

Time (Hours)	Cumulative % Drug Release					
	Formulation Code					
	F4 (D:P 1:4)		F5 (D:P 1:1)		F6 (D:P 1:2)	
	Mean	±SD	Mean	±SD	Mean	±SD
0.25	5.625	1.546619	11.3625	1.0125	11.7375	2.571144
0.5	12.8125	0.735335	14.71375	0.760048	14.23083	1.267969
0.75	14.97917	0.742995	17.57583	0.81698	17.0875	1.097124
1	17.84333	0.516366	21.29292	0.584883	19.29917	0.799446
1.5	20.55	0.518623	24.56167	1.573119	20.33333	1.42111
2	22.27208	1.030043	27.37667	2.054021	22.38917	0.990835
2.5	23.18583	0.594645	27.3325	1.915728	24.16542	0.501841
3	24.81958	0.92565	30.17208	0.591091	25.77125	0.802632
3.5	27.29375	0.364865	33.49	1.053218	27.39208	0.802944
4	29.905	0.473483	34.89125	0.471671	28.87792	0.800212
4.5	31.75458	0.875049	36.75417	0.465833	30.86458	0.444438
5	34.25875	1.340195	38.06	0.989207	31.52	0.3675
5.5	36.63708	0.801224	39.73613	1.132155	33.67917	0.70902
6	38.85042	1.020595	41.82779	1.159991	35.37125	0.530729
6.5	40.25917	1.074487	45.03321	0.983941	37.26583	0.528894
7	41.86667	0.396521	47.05454	0.608796	39.14	0.541808
7.5	43.4875	0.350475	49.73829	1.353056	40.69333	0.644765
8	45.90917	0.661369	52.59571	1.431443	42.97208	0.553513
8.5	47.90292	0.87578	56.51871	1.884152	44.93417	0.551561
9	51.22625	1.599676	60.15496	1.131806	47.21333	0.859706
9.5	53.08125	0.932814	62.58788	1.631339	49.85042	1.073261
10	55.13875	0.596884	65.90371	0.989954	53.89917	1.448386
10.5	57.02583	0.889507	68.91204	1.893726	57.95	0.999629
11	58.92792	0.857519	72.09663	1.403315	62.115	1.104165
11.5	60.17	2.045867	77.10913	0.549071	69.2825	1.284288
12	63.63208	1.24939	82.24454	0.11861	76.26042	0.839038

Table – 3b

In Vitro Release Profile of Perindopril Erbumine Floating Microspheres

Time (Hours)	Cumulative % Drug Release					
	Formulation Code					
	F7(D:P 1:3)		F8(D:P 1:4)		F9 (D:P 1:1)	
	Mean	±SD	Mean	±SD	Mean	±SD
0.25	11.4	0.507291	6.15	2.424194	9.1875	2.041599
0.5	13.55167	0.509489	12.29333	0.822168	14.20208	1.259966
0.75	16.02583	0.589314	14.79167	1.436209	17.54625	0.826284
1	19.08833	0.762185	18.17875	1.10406	20.92583	1.022049
1.5	20.64542	0.529101	20.36417	0.555572	23.02833	0.842134
2	23.905	0.523038	22.49667	0.611305	26.16417	1.097106
2.5	25.17333	0.528669	24.08792	1.061456	28.61958	0.862559
3	26.94042	1.03779	25.69417	0.647102	31.62417	1.386895
3.5	28.76167	0.929286	27.61542	1.388316	34.65875	1.391016
4	30.6	0.551403	29.5925	0.82516	38.21083	1.64748
4.5	32.75542	0.245949	32.26333	0.532703	41.01083	0.916565
5	34.25625	1.063249	33.94792	1.329334	44.1	0.701574
5.5	34.6075	1.031223	35.985	0.608134	44.89375	1.519839
6	37.17125	0.813819	38.04083	0.433246	48.24125	0.633765
6.5	39.57208	0.79889	40.26542	1.123544	48.92083	1.168885
7	41.65792	0.878196	41.53542	0.315011	48.59	0.481205
7.5	43.6125	0.837149	43.64	0.628126	49.7125	0.490453
8	47.75917	0.409276	45.42583	0.920096	51.55458	1.166697
8.5	49.05958	0.18657	46.88917	0.92895	53.08542	0.652112
9	55.95667	1.605639	49.03875	0.651254	55.28013	0.409143
9.5	57.525	2.122337	51.05708	0.991026	56.67971	0.830501
10	60.82958	0.630993	53.5425	1.171762	60.93888	0.624293
10.5	63.41458	1.341159	56.275	1.787773	67.30179	4.292064
11	68.75917	1.624908	59.51958	1.677128	69.90388	0.710502
11.5	72.95625	1.132812	63.46917	0.956174	74.29013	1.299974
12	74.5675	1.406986	68.13125	0.935784	77.78013	1.227609

Table – 3c

In Vitro Release Profile of Perindopril Erbumine Floating Microspheres

Time (Hours)	Cumulative % Drug Release					
	Formulation Code					
	F10 (D:P 1:2)		F11 (D:P 1:3)		F12 (D:P 1:4)	
	Mean	±SD	Mean	±SD	Mean	±SD
0.25	7.1625	1.804681	6.825	1.856534	8.1	1.14175
0.5	13.50458	1.779342	14.32583	1.252112	12.84083	0.333158
0.75	16.65375	1.020694	17.18417	0.528368	16.17	0.498549
1	20.73625	0.795378	16.9975	4.59429	17.88458	0.50567
1.5	22.98708	0.804243	19.73167	1.2035	19.91625	0.271733
2	25.93542	1.80723	21.81917	0.540012	22.15583	1.031917
2.5	26.55125	1.937434	23.74	0.376889	23.74292	0.743759
3	28.33333	1.606949	25.34208	0.560186	25.195	0.749741
3.5	30.50708	0.826908	27.48417	1.125247	26.62292	1.800435
4	32.70167	0.449549	30.13458	0.871343	28.58875	1.642586
4.5	34.54208	0.537623	31.42375	1.249402	30.57333	1.082585
5	36.77417	0.794396	33.28625	0.843915	32.08917	0.518562
5.5	37.63958	1.222985	35.50333	0.608324	34.25583	1.101449
6	40.38542	1.073985	38.37875	0.587145	36.48042	1.311715
6.5	43.1575	0.578418	41.13208	0.344783	37.86333	1.325541
7	45.28083	0.836303	43.23667	0.396883	39.745	1.148176
7.5	47.08542	1.009644	44.8725	0.204393	42.01833	1.624407
8	49.2425	0.855686	44.79667	1.764035	43.9375	1.43792
8.5	51.41833	1.160954	47.0775	0.94575	45.87333	1.881417
9	53.61292	1.038901	49.41625	0.171469	48.53833	1.201989
9.5	56.16375	0.631977	50.76333	0.184706	51.22792	2.176612
10	58.54958	0.728992	52.7575	0.416719	53.90333	2.041307
10.5	60.73083	0.462941	55.14333	0.468362	58.16792	1.918457
11	64.13	0.946203	59.2	0.486486	62.11167	2.484164
11.5	69.13542	0.660611	64.60833	2.397258	65.60542	2.925142
12	72.65208	0.941215	71.68292	0.628617	69.46875	0.367553

Table – 3d

In Vitro Release Profile of Perindopril Erbumine Floating Microspheres

Time (Hours)	Cumulative % Drug Release					
	Formulation Code					
	F13 (D:P 1:1)		F14 (D:P 1:2)		F15 (D:P 1:3)	
	Mean	±SD	Mean	±SD	Mean	±SD
0.25	9.493333	2.600276	14.98	0.52144	14.17	1.102588
0.5	11.95333	2.362717	15.69333	0.793872	15.03333	1.368442
0.75	14.95333	1.199264	17.81667	1.068285	17.03667	1.605314
1	17.65	1.998274	18.24667	1.26263	18.42	0.536936
1.5	19.86667	0.851724	19.35333	1.143431	19.65667	0.411987
2	21.92333	1.576843	20.55667	0.688791	21.34667	0.930287
2.5	24.03	0.904212	22.20667	0.677963	22.81333	1.298666
3	25.56333	1.167576	22.52	1.230894	23.93667	1.459086
3.5	27.98333	2.888968	24.60333	0.811316	25.88667	0.786914
4	28.36	2.715345	26.73333	0.735822	27.72333	1.537606
4.5	30.05	2.624633	26.60333	1.718847	29.55667	2.037163
5	32.02	3.473197	28.27667	1.160704	31.6	0.843267
5.5	33.55333	3.522504	29.09333	1.115542	33.45	1.261467
6	38.07667	3.689679	31.07333	1.120952	32.59333	3.30155
6.5	38.72667	3.756891	32.05333	0.300056	34.28	3.354236
7	42.71	2.816931	35.49667	1.487425	36.23	3.917895
7.5	44.65667	3.302822	40.62	1.88008	42.13667	0.748086
8	51.51	3.761396	42.88	0.870689	44.48667	1.372747
8.5	54.52667	3.960067	47.57	2.175017	48.04	0.727461
9	57.46	0.736546	54.75	3.902461	51.64333	1.796228
9.5	63.70667	1.808462	57.16	1.872618	55.98	2.732105
10	68.22	3.412858	60.67333	2.223181	60.61667	2.708733
10.5	73.16667	1.651706	64.70667	0.260832	66.53	2.58215
11	76.28333	2.283689	69.22667	1.392276	69.69667	3.865751
11.5	79.88333	1.037224	71.66333	2.104954	69.86333	2.530856
12	82.11333	1.445764	73.30333	1.768738	72.86	1.998274

Table – 3e

In Vitro Release Profile of Perindopril Erbumine Floating Microspheres

Time (Hours)	Cumulative % Drug Release					
	Formulation Code					
	F16 (D:P 1:4)		F17 (D:P 1:1)		F18 (D:P 1:2)	
	Mean	±SD	Mean	±SD	Mean	±SD
0.25	5.96	1.548257	10.36	2.565015	18.11	1.548257
0.5	12.28667	0.810946	14.88667	2.389756	21.68467	1.03201
0.75	15.46333	1.272255	22.28667	0.536128	24.13667	0.768266
1	16.79333	1.050016	25.90667	1.300936	27.98	0.25
1.5	18.51	0.9531	29.41667	0.478992	28.95333	1.465754
2	19.23667	0.446132	32.24333	1.811307	30.80533	2.819593
2.5	21.28	0.264575	36.30333	0.810267	32.81	1.253834
3	22.67	1.045897	39.89333	0.381357	34.85	1.261943
3.5	23.24333	1.609358	42.97	1.080879	38.1	1.992712
4	24.49667	1.624633	44.72667	1.114286	42.04	1.775021
4.5	26.29333	1.125093	46.79667	3.071845	45.49667	0.761993
5	27.38667	1.26753	49.14	2.174695	47.82667	2.051008
5.5	29.37333	1.702273	54.33	2.707342	50.22	1.84258
6	31.19	1.751942	56.62	2.510598	52.53667	0.53799
6.5	32.18	1.464616	59.31667	1.261837	55.4	1.481317
7	33.66667	1.320391	63.32333	2.876549	59.25	1.036774
7.5	35.20667	1.220341	65.94667	2.558913	60.31333	1.007588
8	36.75333	4.032088	69.02333	1.998032	63.31333	1.620041
8.5	40.49333	2.278296	70.51733	0.715039	66.11	0.571052
9	44.77667	2.701672	72.72667	0.820386	67.08667	1.235084
9.5	48.04667	2.948446	74.86	0.521632	68.80667	0.166233
10	51.22	2.960203	78.63	0.347707	70.59667	0.956312
10.5	55.06667	2.952581	80.37333	0.792233	73.09667	0.673226
11	60.74	4.296894	83.7	1.160129	74.88	0.390512
11.5	66.89333	1.017955	85.82667	0.217332	75.86333	1.025736
12	68.58333	2.567729	86.95333	0.633193	78.05333	0.241109

Table – 3f

In Vitro Release Profile of Perindopril Erbumine Floating Microspheres

Time (Hours)	Cumulative % Drug Release			
	Formulation Code			
	F19 (D:P 1:3)		F20 (D:P 1:4)	
	Mean	±SD	Mean	±SD
0.25	6.483333	1.304697	5.516667	0.695869
0.5	15.14333	1.546167	12.43	1.005037
0.75	16.85	1.313278	15.87667	0.487887
1	20.89333	1.010412	19.68	1.253675
1.5	23.13667	0.44658	21.80333	1.45139
2	24.63	0.554617	24.03	0.832646
2.5	28.55	1.655023	27.28667	0.570468
3	29.88667	0.833627	29.45333	0.551483
3.5	33.48667	1.209683	31.76667	1.372455
4	35.56667	0.866333	34.46667	0.905612
4.5	38.15	1.105848	35.48667	1.434376
5	38.88667	2.40134	35	2.157869
5.5	42.49	1.557915	37.38667	1.361225
6	44.61	1.774345	39.11	1.185791
6.5	46.42667	0.570029	41.41667	0.925869
7	48.42333	0.46694	43.03667	0.595511
7.5	50.92667	1.557573	44.57	1.348369
8	54.10333	0.785133	45.40667	0.651178
8.5	56.67667	1.558728	46.52333	0.90257
9	58.97667	1.706878	48.84667	1.080293
9.5	60.83667	1.600292	50.28	0.886397
10	64.81	1.135297	53.33	2.798339
10.5	66.81	1.731127	56.72667	1.516718
11	69.74333	0.150444	60.35667	0.966713
11.5	71.70667	0.285715	63.55667	2.711205
12	73.36667	0.255016	66.84667	2.26014

Table 4: Percentage Yield, Particle Size, Entrapment Efficiency and Percentage Buoyancy.

Formulation code	Theoretical loading (%)	Actual loading (%)	Percentage yield (%)	Particle size(µm)	Entrapment efficiency (%)	Percentage buoyancy (%)
F1	50.00	42.05	76.27	521.3	84.10	62.06
F2	33.33	29.43	65.54	435.0	88.29	65.30
F3	25.00	22.89	66.15	430.0	91.58	66.07
F4	20.00	19.15	75.16	569.0	95.79	69.62
F5	50.00	42.99	74.00	666.0	85.98	68.66
F6	33.33	29.43	69.12	865.2	88.29	71.72
F7	25.00	23.36	83.56	858.0	93.45	77.91
F8	20.00	19.19	76.84	983.0	95.79	82.77
F9	50.00	33.17	66.80	447.8	66.35	66.54
F10	33.33	25.75	78.08	566.6	77.25	67.01
F11	25.00	15.88	79.95	603.6	79.43	69.20
F12	20.00	18.69	71.52	761.3	93.45	67.85
F13	50.00	33.64	80.12	500.2	67.28	68.07
F14	33.33	22.89	64.28	616.0	68.69	70.00
F15	25.00	19.62	71.36	707.4	78.50	72.83
F16	20.00	17.28	60.05	716.6	86.44	73.58
F17	50.00	35.51	78.60	536.7	71.02	66.66
F18	33.33	26.16	72.56	612.0	78.51	68.51
F19	25.00	22.89	75.76	677.0	91.56	71.48
F20	20.00	19.15	91.58	801.0	95.75	74.33

Table-5 KINETICS OF IN-VITRO RELEASE FROM FLOATING MICROSPHERES OF PERINDOPRIL ERBUMINE

Formulation Code	Zero order Kinetics		First order Kinetics		Higuchi Model		Korsmeyer-Peppas model		Hixson Crowell	
	R^2 value	$K_0(mg/h^{-1})$	**R^2 value**	$K_1(h^{-1})$	**R^2 value**	$K_H(mg/h^{-1})$	R^2 value	**n value**	**R^2 value**	$K_{HC}(h^{-1/3})$
FI	0.9640	5.784	0.9799	-0.0537	0.9860	23.05	0.9823	0.5276	0.9911	-0.144
F2	0.9606	5.262	0.9804	-0.0430	0.9894	20.93	0.9876	0.5186	0.9929	-0.122
F3	0.9846	4.617	0.9662	-0.0314	0.9615	18.45	0.9719	0.6427	0.9841	-0.097
F4	0.9755	4.506	0.9813	-0.0310	0.9773	18.03	0.9772	0.5818	0.9916	-0.093
F5	0.9745	5.478	0.9035	-0.0466	0.9385	21.59	0.9422	0.5888	0.9458	-0.129
F6	0.9474	4.606	0.9851	-0.0347	0.8824	17.88	0.9181	0.5941	0.8931	-0.100
F7	0.9714	5.101	0.9142	-0.0399	0.9172	20.02	0.9365	0.6228	0.9419	-0.114
F8	**0.9879**	4.544	**0.9600**	-0.0319	**0.9973**	18.11	0.9698	**0.5765**	**0.9740**	-0.045
F9	0.9570	5.142	0.9326	-0.0413	0.9670	20.54	0.9732	0.5509	0.9561	-0.116
F10	0.9700	4.857	0.9536	-0.0365	0.9619	19.30	0.9594	0.5556	0.9710	-0.106
F11	0.9683	4.573	0.9320	-0.0327	0.9483	18.13	0.9685	0.5957	0.9553	-0.096
F12	0.9720	4.606	0.9336	-0.0330	0.9342	18.16	0.9487	0.5992	0.9551	-0.097
F13	0.9690	5.953	0.8775	-0.0579	0.8902	23.47	0.9223	0.7266	0.9158	-0.143
F14	0.9359	5.038	0.8647	-0.0393	0.8261	19.33	0.8795	0.6982	0.8909	-0.113
F15	0.9524	5.000	0.8844	-0.0387	0.8630	19.33	0.9095	0.6688	0.9114	-0.111
F16	0.9780	6.007	0.9846	-0.0518	0.9847	24.33	0.9878	0.6302	0.9933	-0.143
F17	0.9655	6.309	0.9722	-0.0649	0.9901	25.35	0.9905	0.5482	0.9908	-0.167
F18	0.9575	5.330	0.9813	-0.0471	0.9814	20.92	0.9873	0.5257	0.9969	-0.128
F19	0.9754	5.270	0.9779	-0.0416	0.9783	21.12	0.9837	0.5839	0.9883	-0.118
F20	0.9508	4.380	0.9574	-0.0306	0.9699	17.51	0.9677	0.5066	0.9666	-0.091

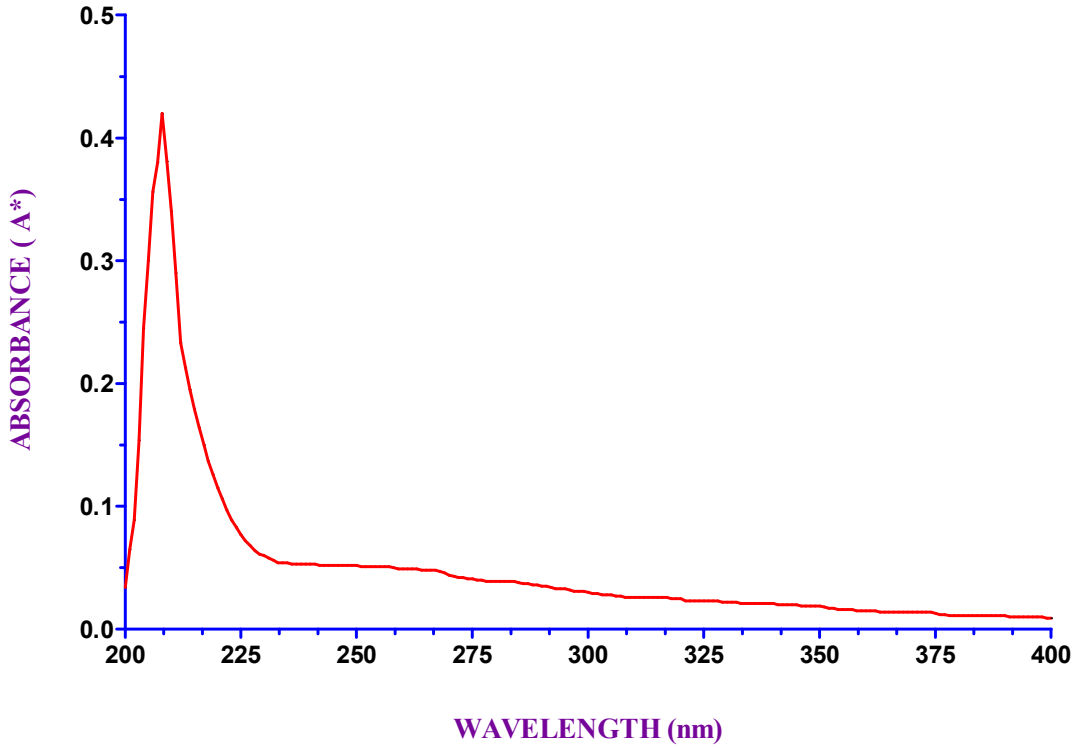


Figure: 14

DETERMINATION OF λ MAX OF PERINDOPRIL ERBUMINE

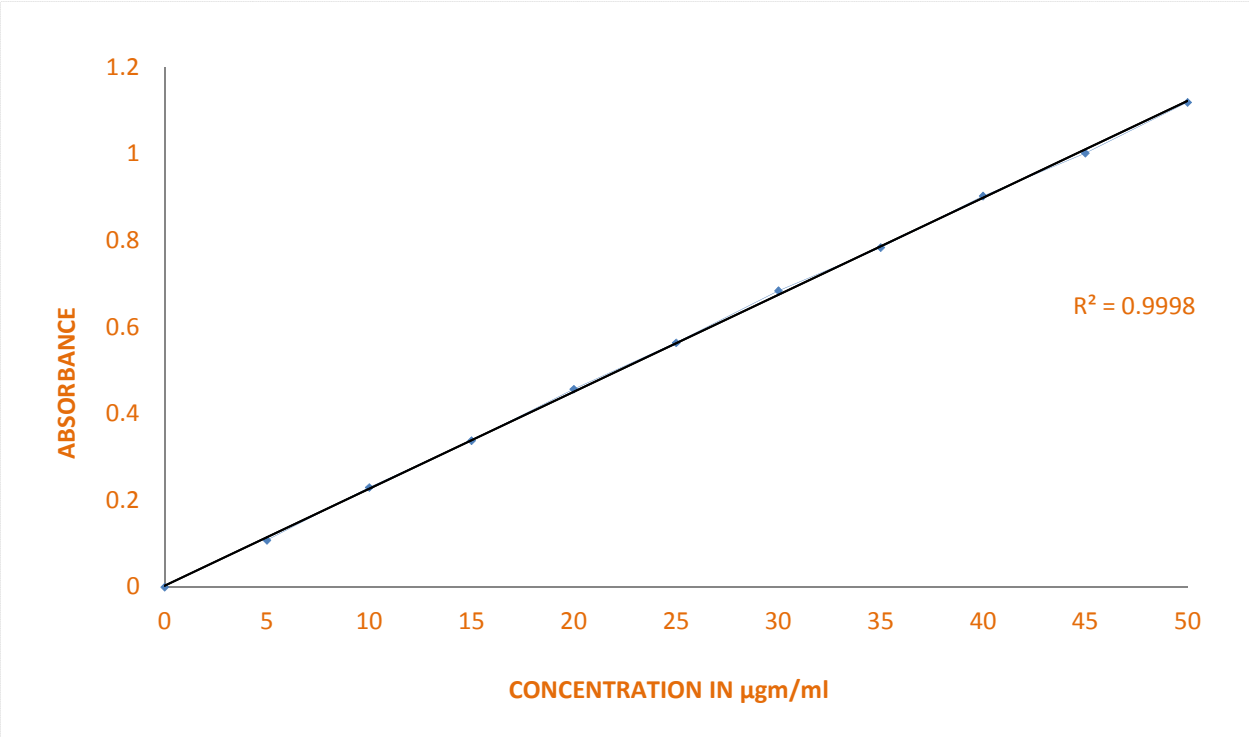
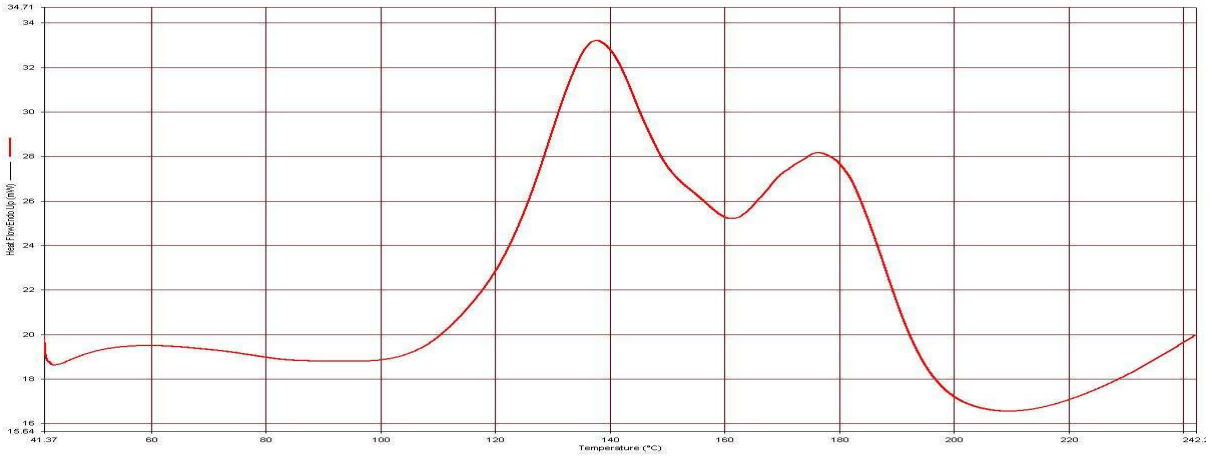


Figure: 15

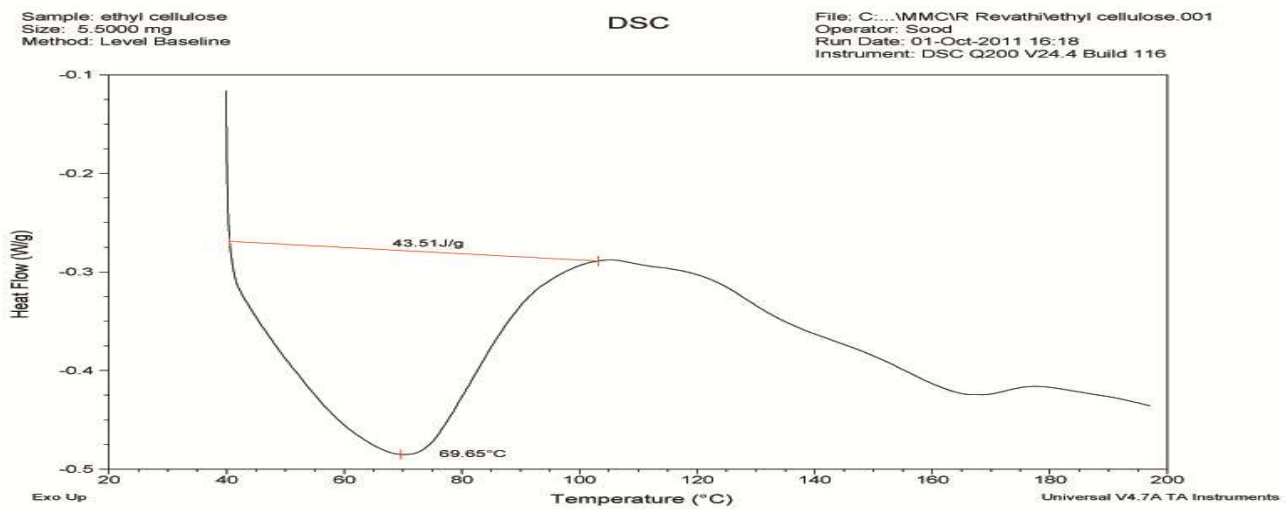
CALIBRATION OF PERINDOPRIL ERBUMINE

Figure 16 DSC THERMOGRAM OF DRUG AND POLYMERS

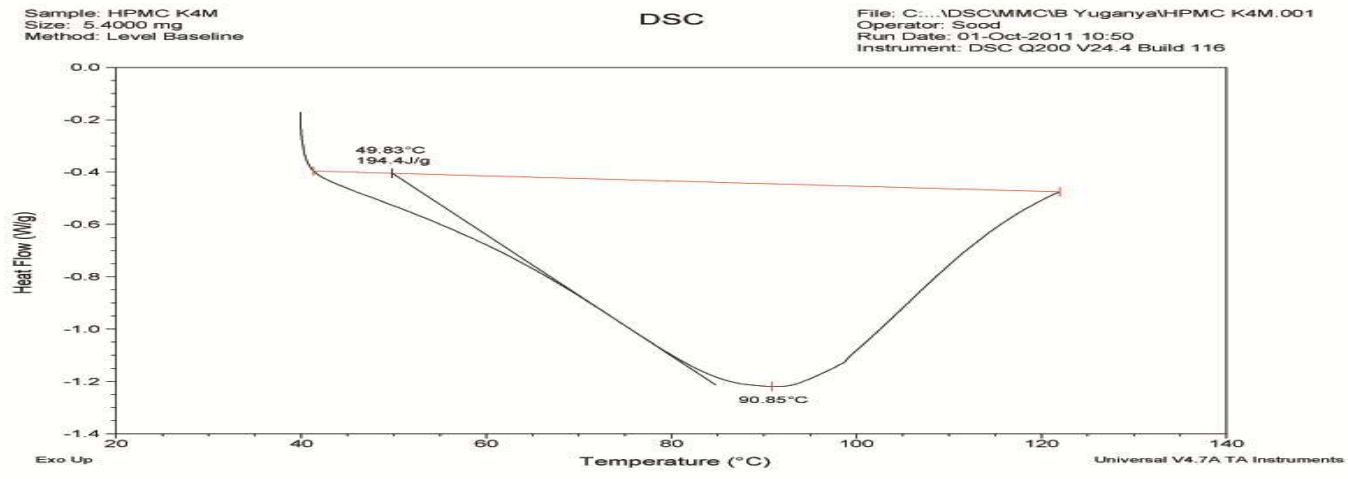
Drug (perindopril erbumine)



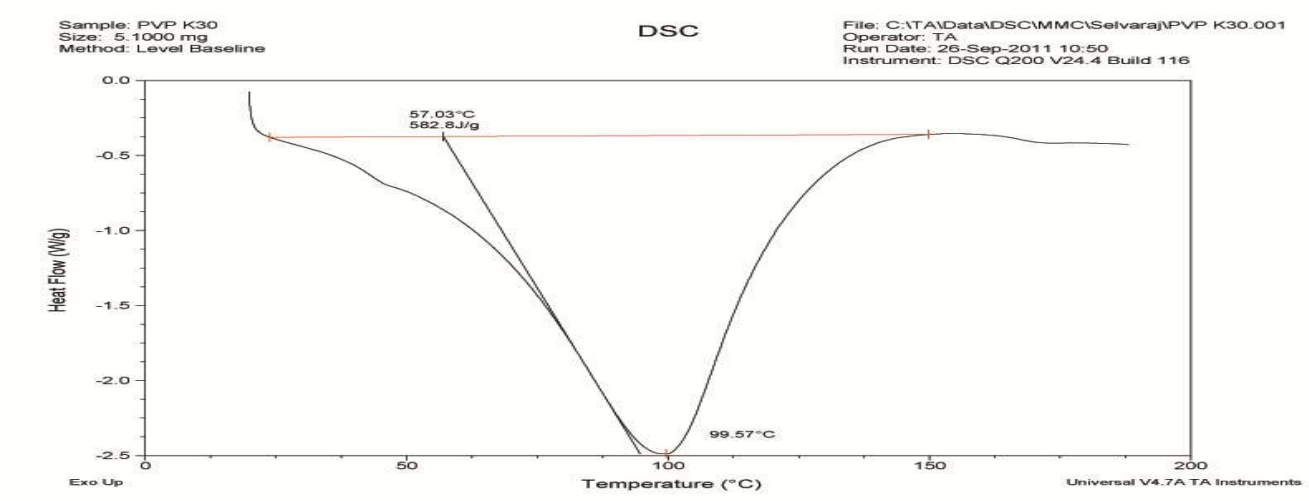
Ethyl cellulose



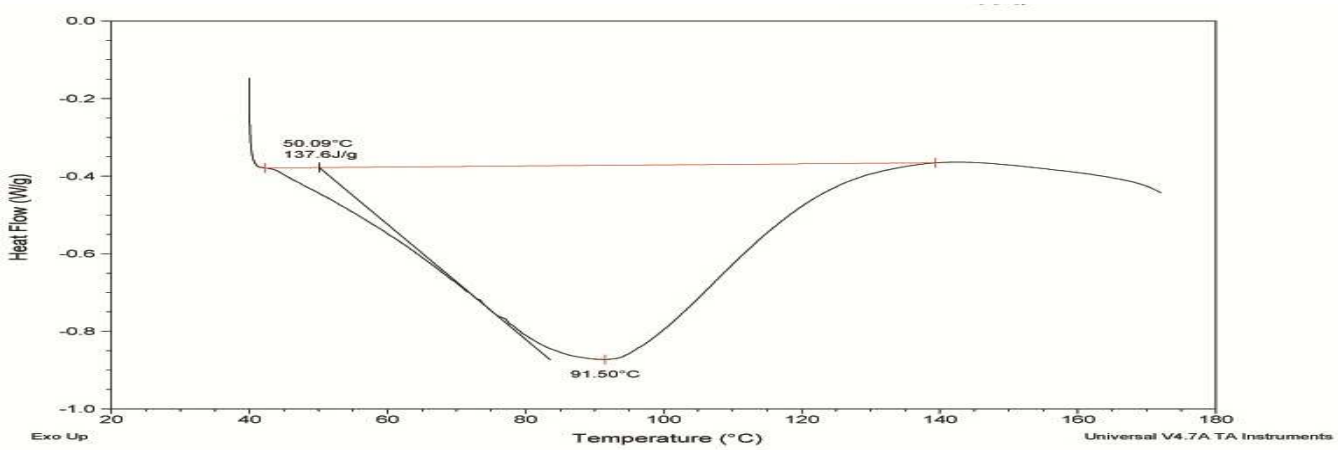
HPMC



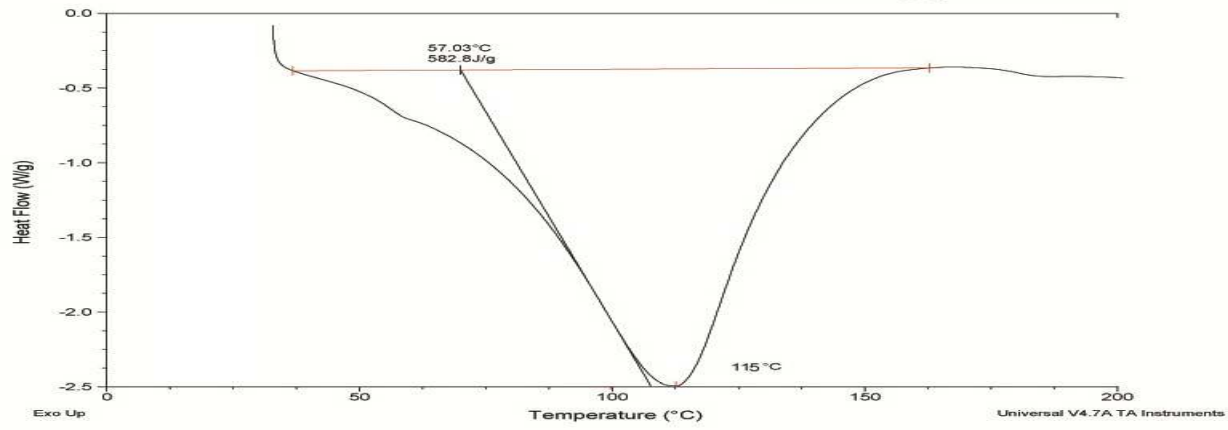
PVP K30



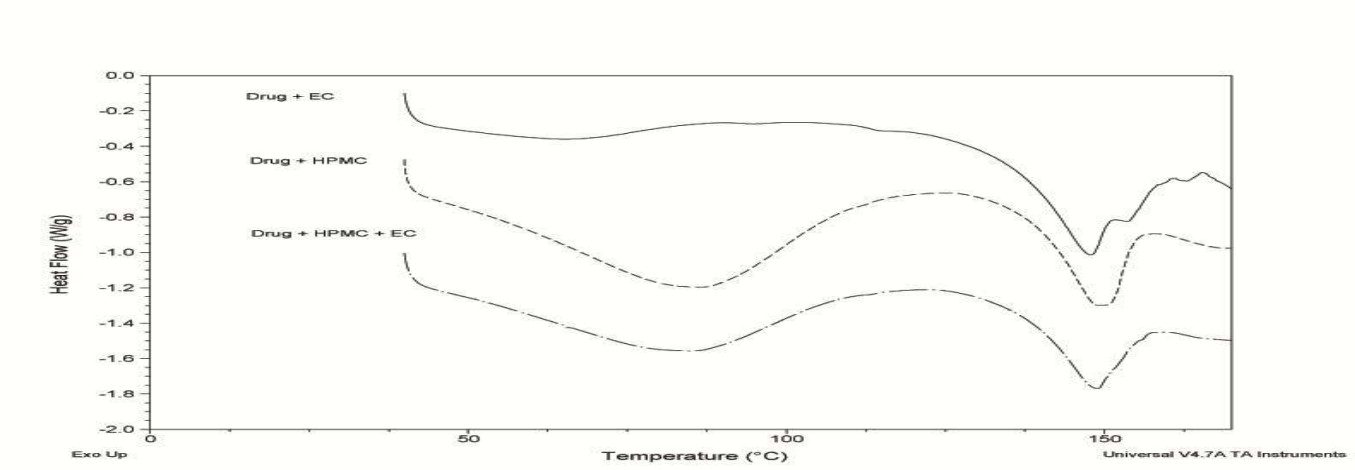
Eudragit S100



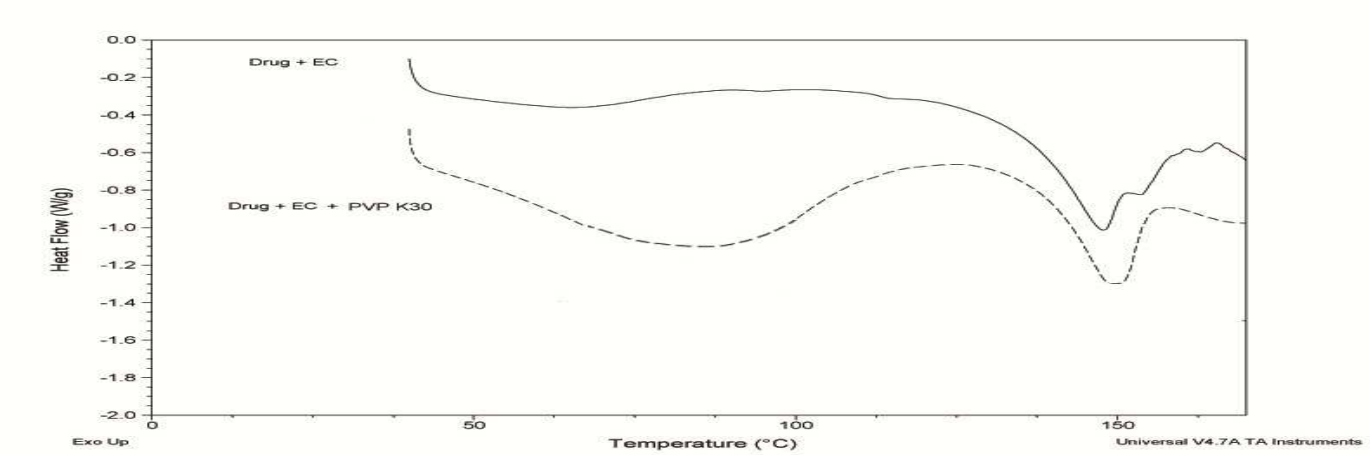
PVP K90



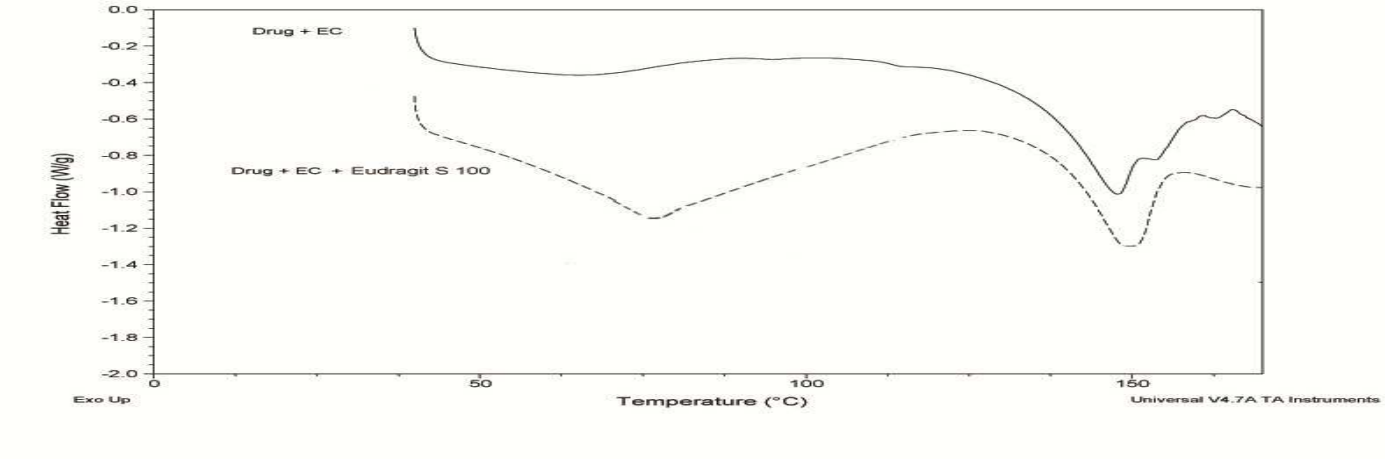
DRUG+EC+HPMC



DRUG+EC+PVP K30



DRUG+EC+ES100



DRUG+EC+PVP K90

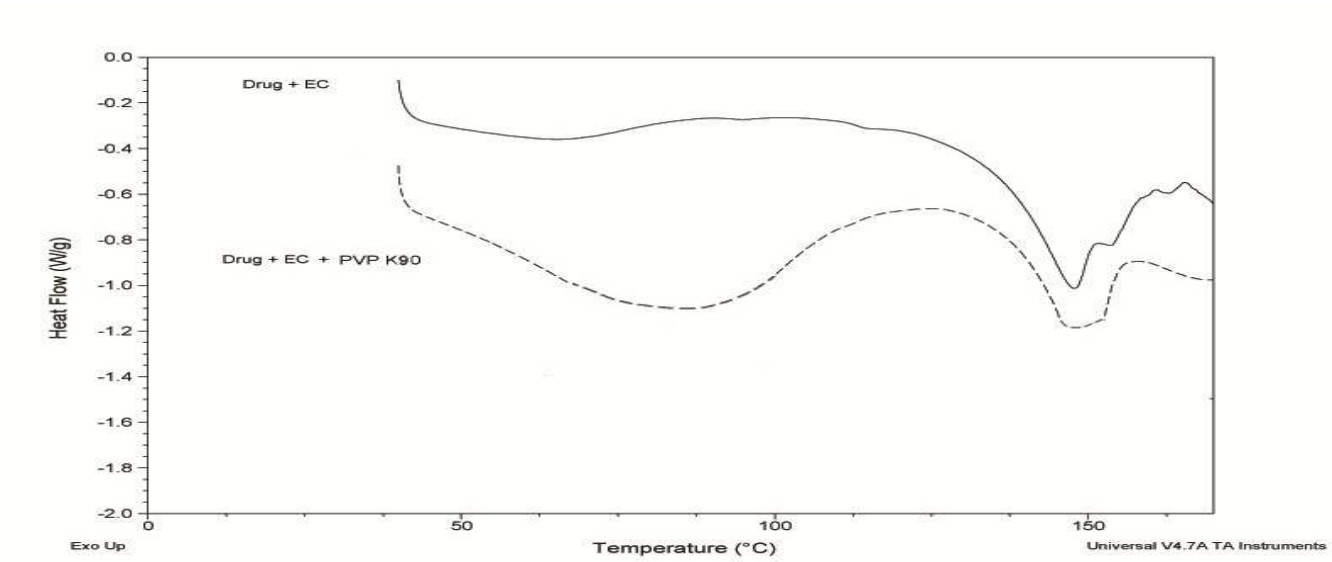
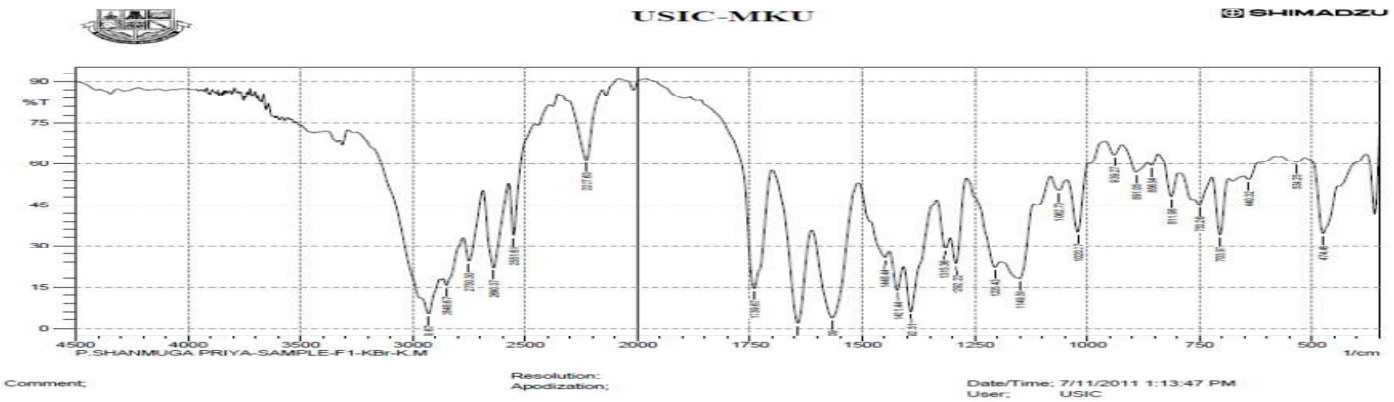
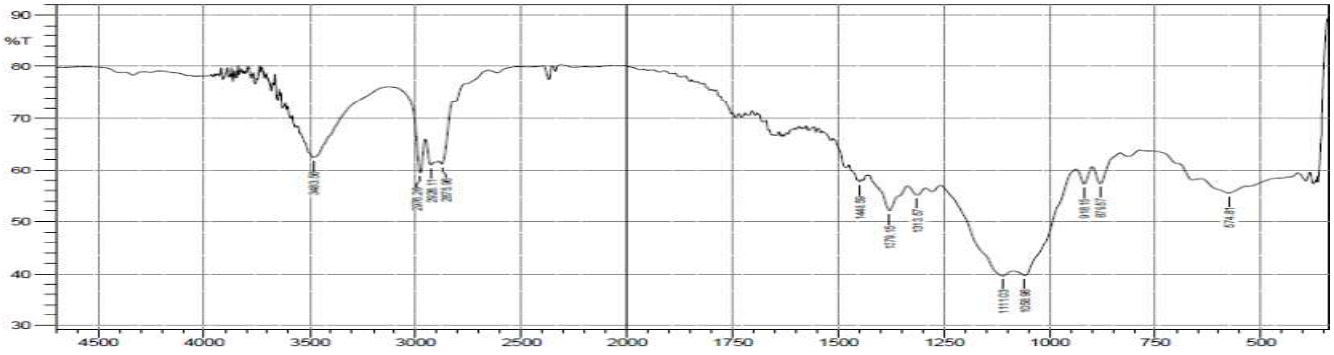

Drug + EC
Drug + EC + PVP K90
Heat Flow (W/g)
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
-1.8
-2.0
0
50
100
150
Temperature (°C)
Exo Up
Universal V4.7A TA Instruments

Figure 17

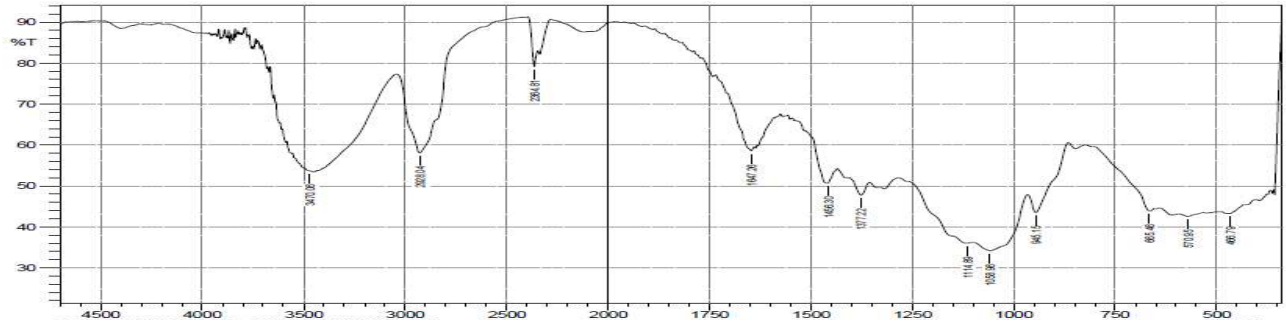
FT-IR SPECRUM OF PERINDOPRIL ERBUMINE



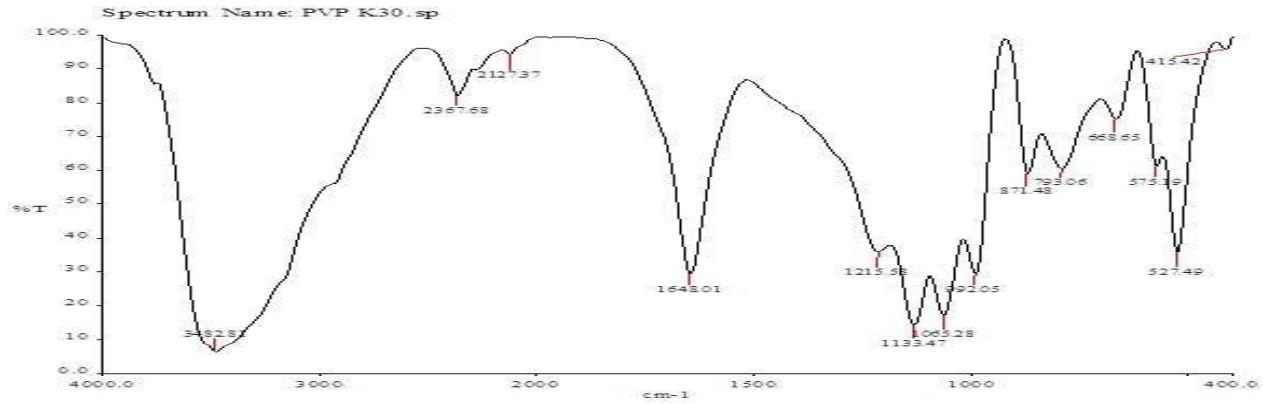
FT-IR SPECRUM OF ETHYL CELLULOSE



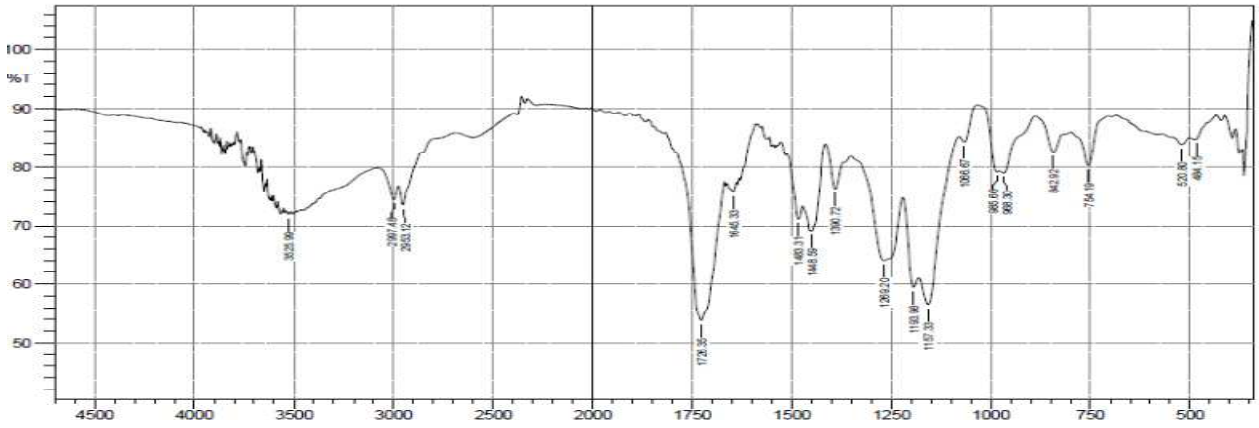
FT-IR SPECRUM OF HPMC



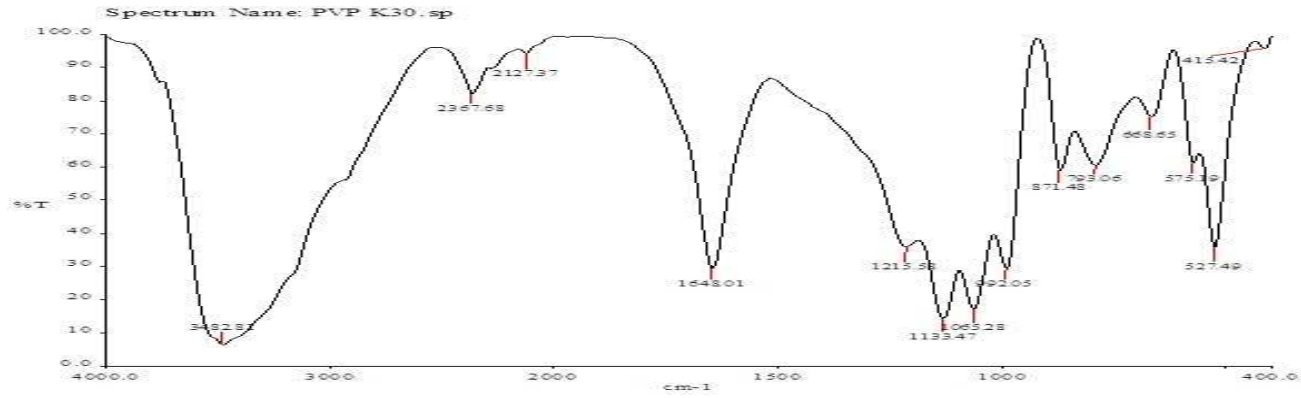
FT-IR SPECRUM OF PVP K30



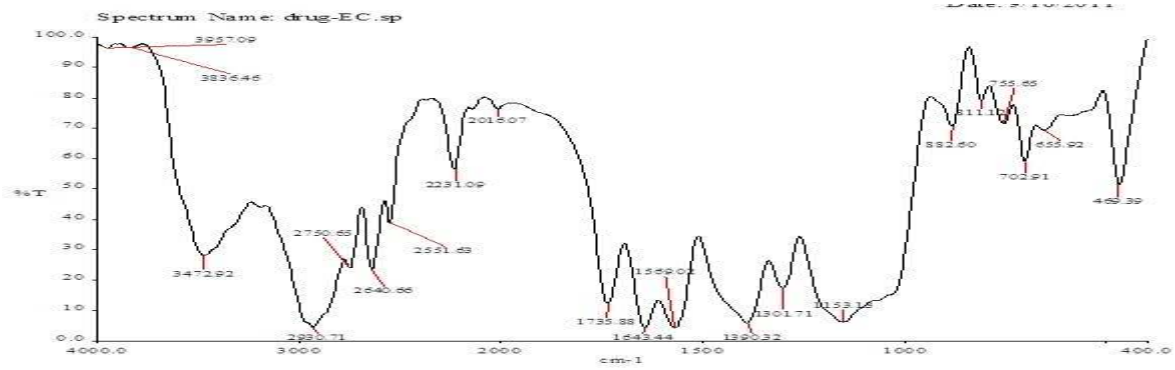
FT-IR SPECRUM OF EUDRAGIT S100



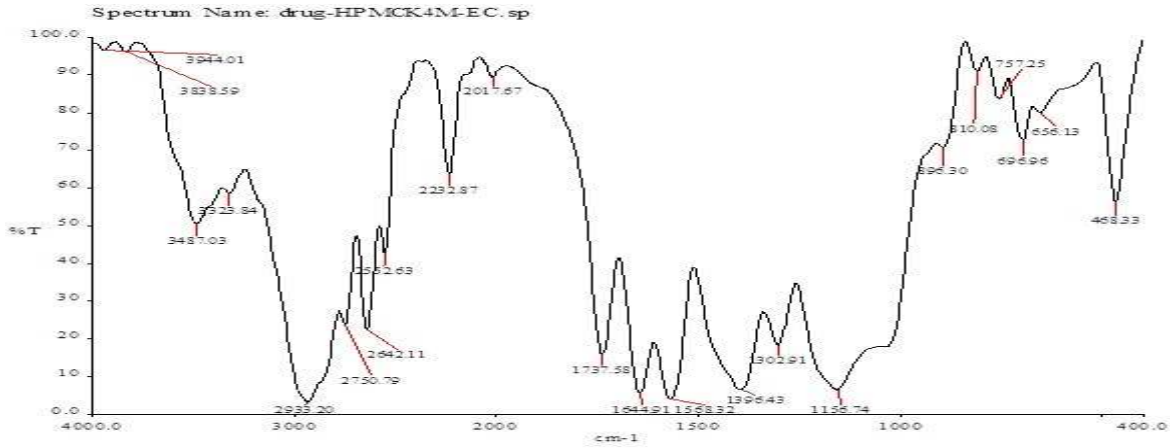
FT-IR SPECRUM OF PVP K90



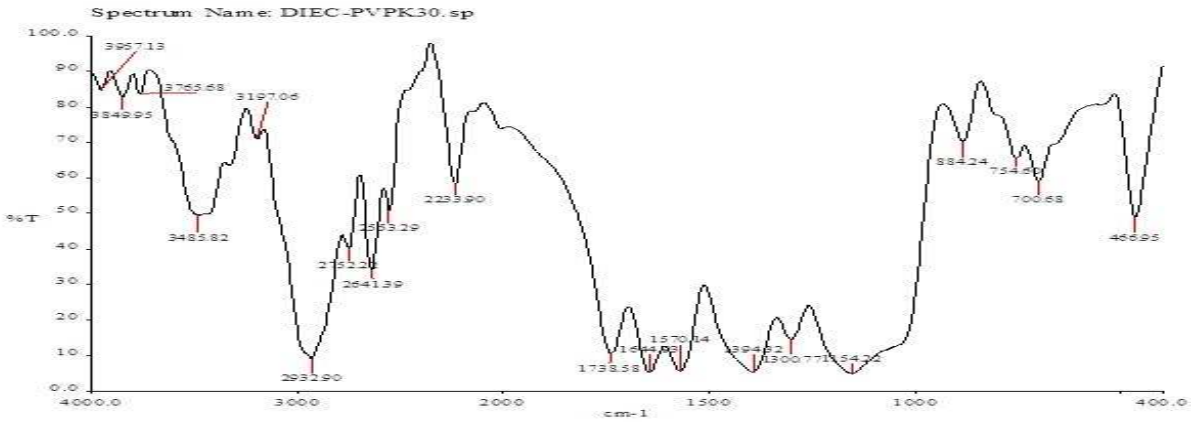
FT-IR SPECRUM OF DRUG+EC



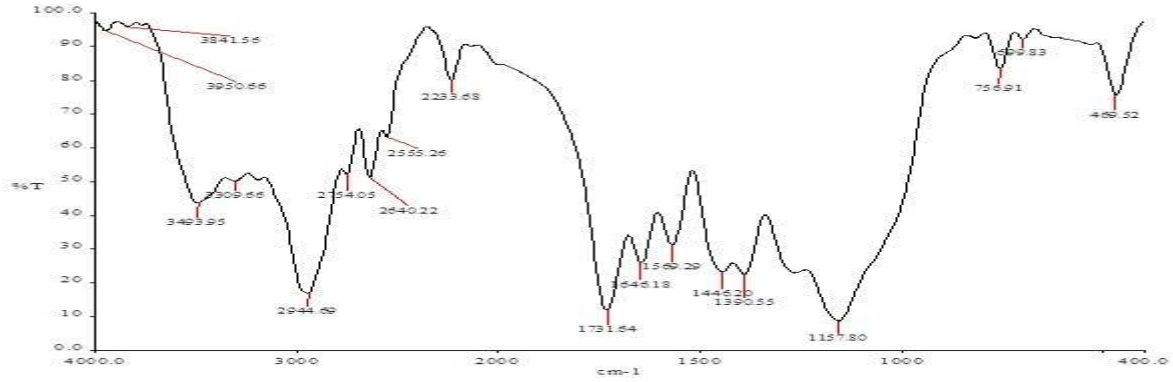
FT-IR SPECRUM OF DRUG+EC+HPMC



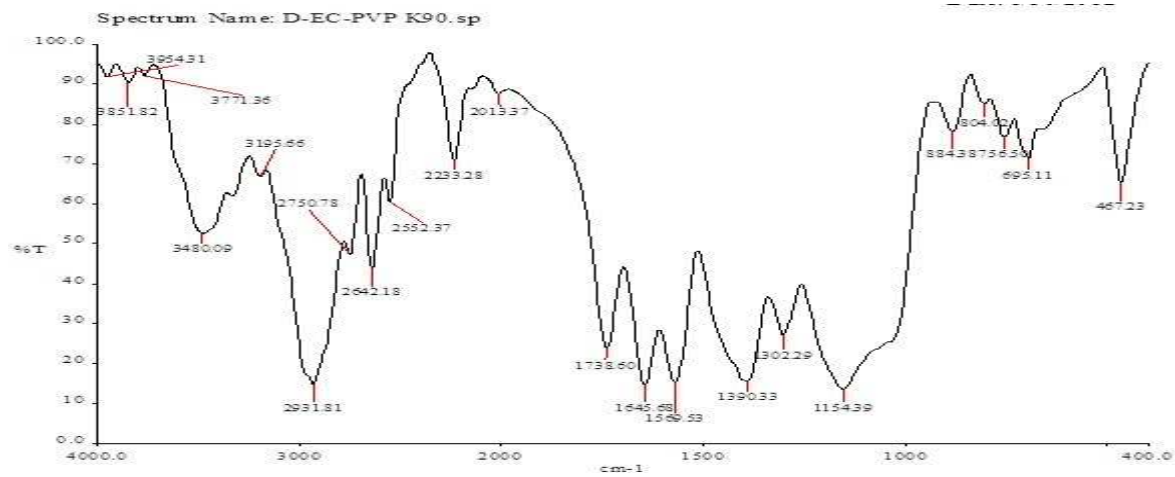
FT-IR SPECRUM OF DRUG+EC+PVP K30



FT-IR SPECRUM OF DRUG+EC+ES100



FT-IR SPECRUM OF DRUG+EC+PVP K90



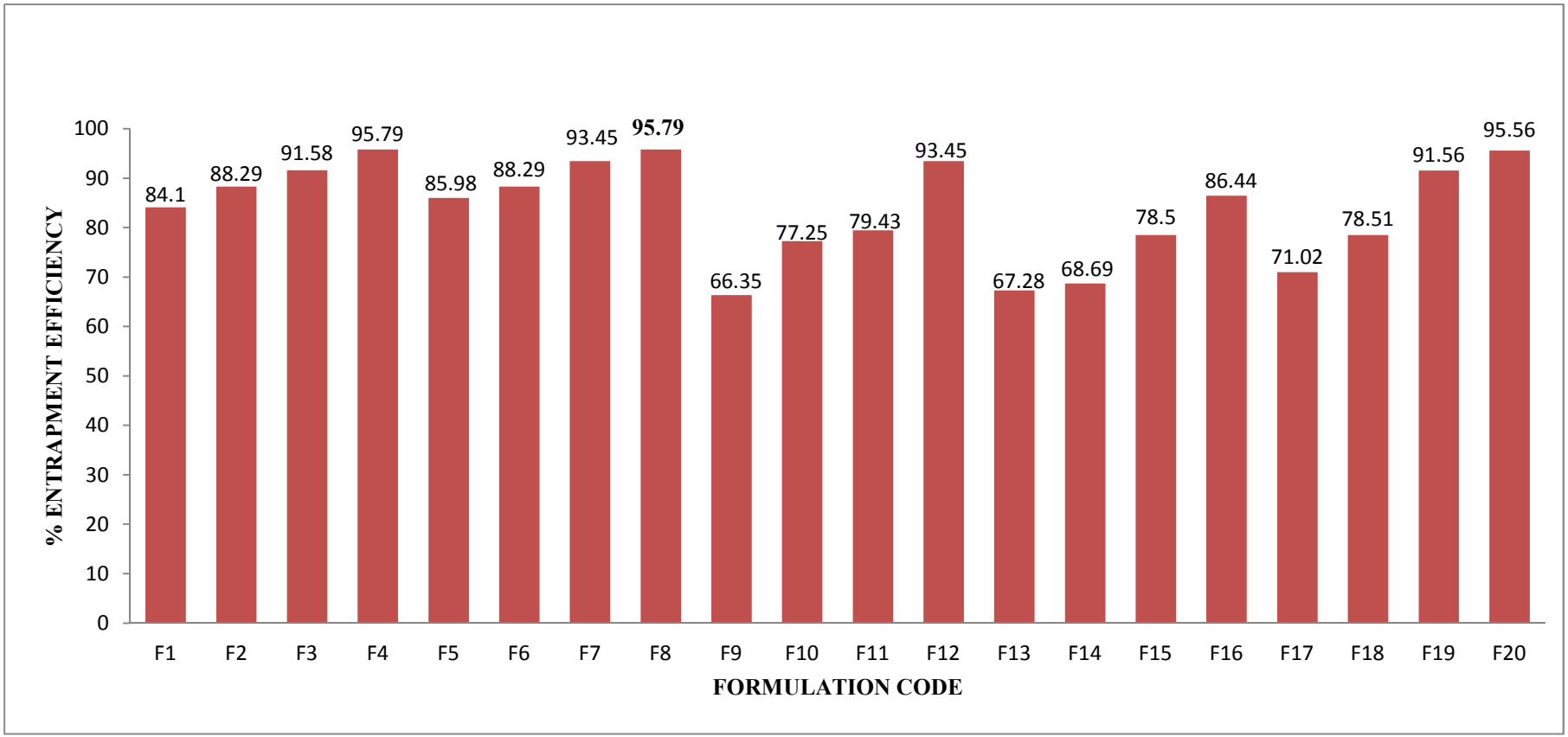


Figure-18

HISTOGRAM OF PERCENTAGE ENTRAPMENT EFFICIENCY OF DIFFERENT PERINDOPRIL ERBUMINE FLOATING MICROSPHERES FORMULATIONS

Figure 19 In-*vitro* floating behaviour of perindopril erbumine floating microspheres

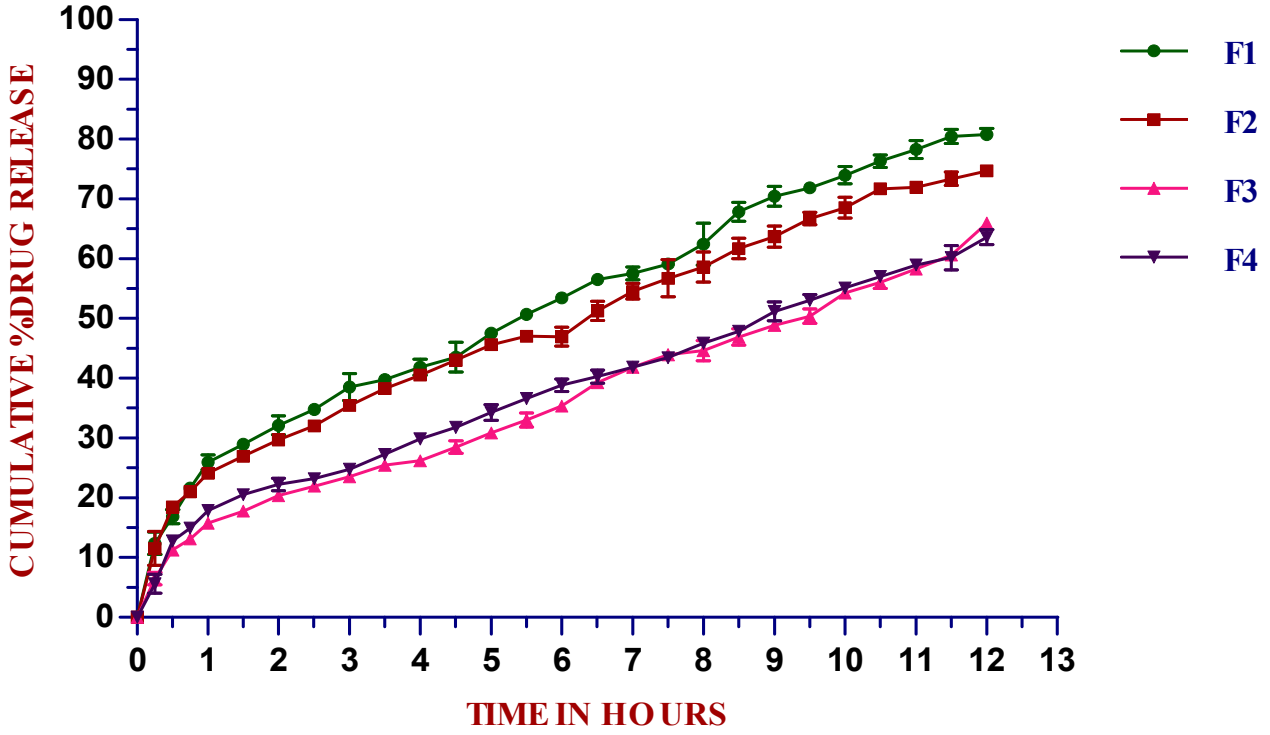


Figure-20

IN-VITRO RELEASE PROFILE OF PERINDOPRIL ERBNUMINE WITH ETHYL CELLULOSE POLYMER AT DIFFERENT RATIOS

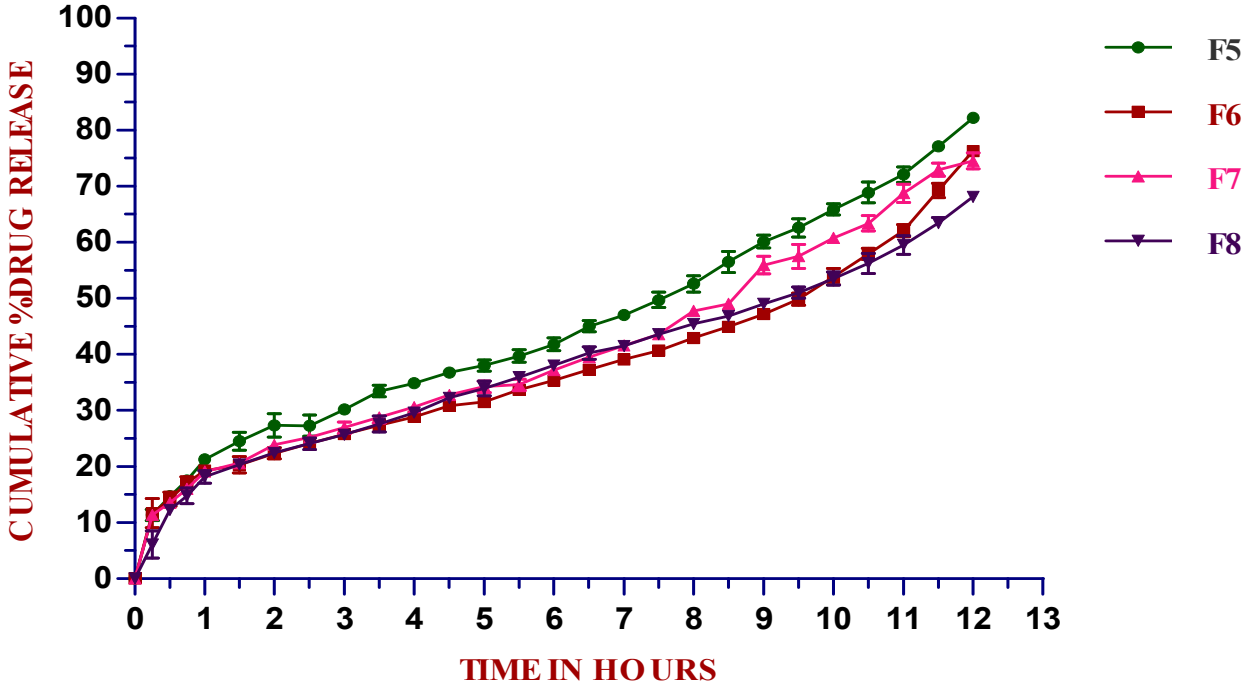


Figure-21

IN-VITRO RELEASE PROFILE OF PERINDOPRIL ERBNUMINE WITH ETHYL CELLULOSE & HPMC POLYMER AT DIFFERENT RATIOS

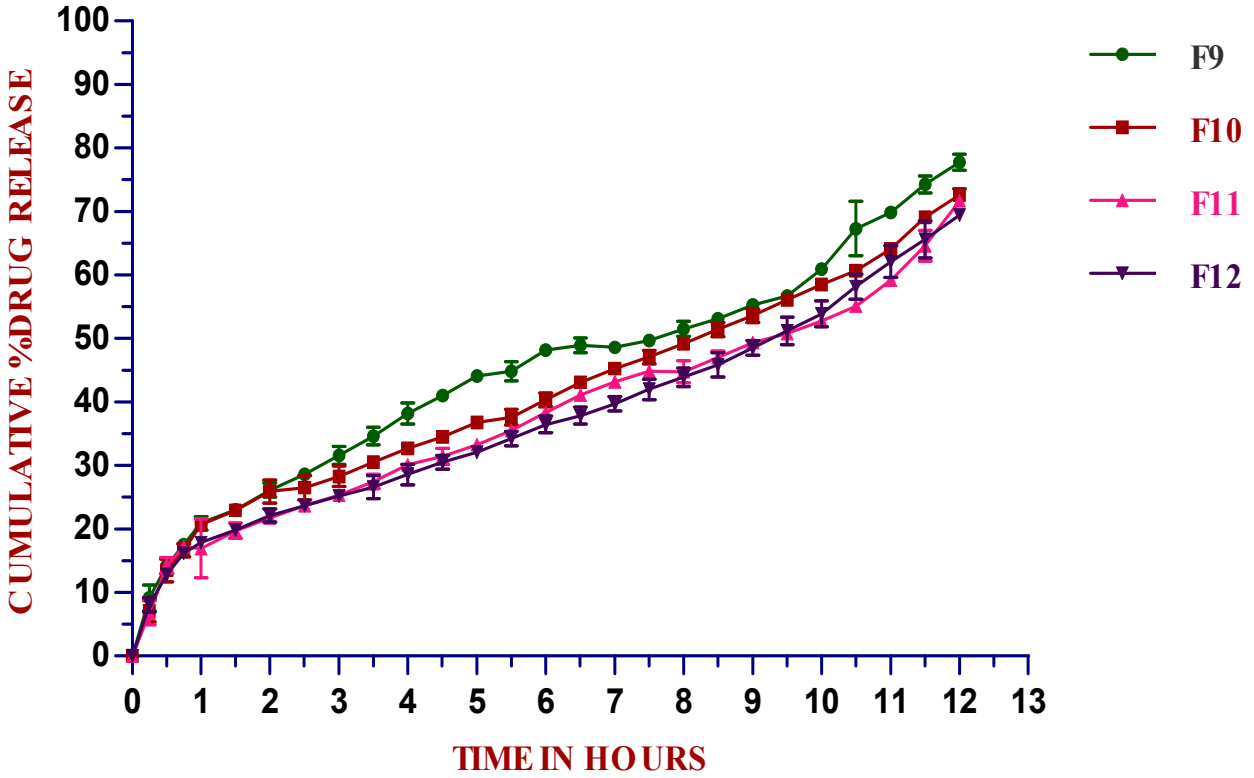


Figure-22

IN-VITRO RELEASE PROFILE OF PERINDOPRIL ERBNUMINE WITH ETHYL CELLULOSE & PVP K30 POLYMER AT DIFFERENT RATIOS

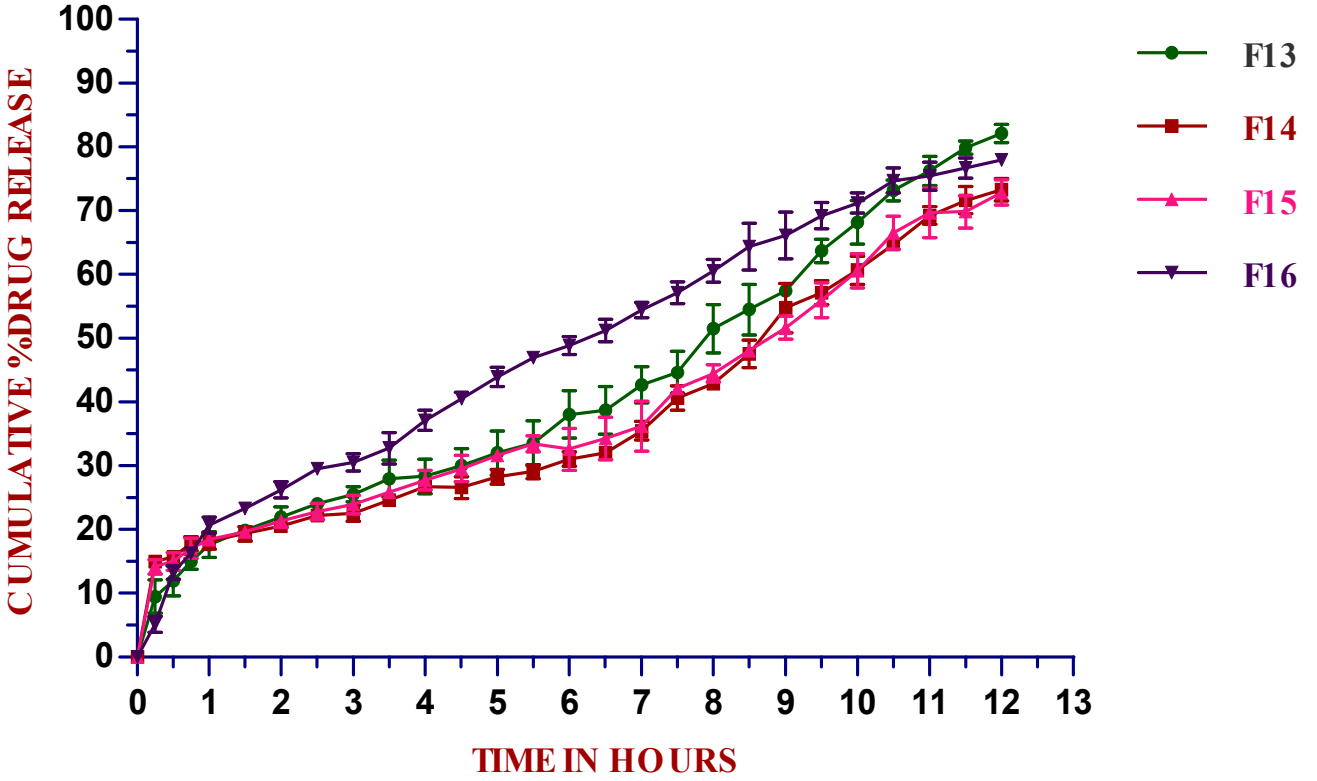


Figure-23

IN-VITRO RELEASE PROFILE OF PERINDOPRIL ERBNUMINE WITH ETHYL CELLULOSE & EUDRAGIT S100 POLYMER AT DIFFERENT RATIOS

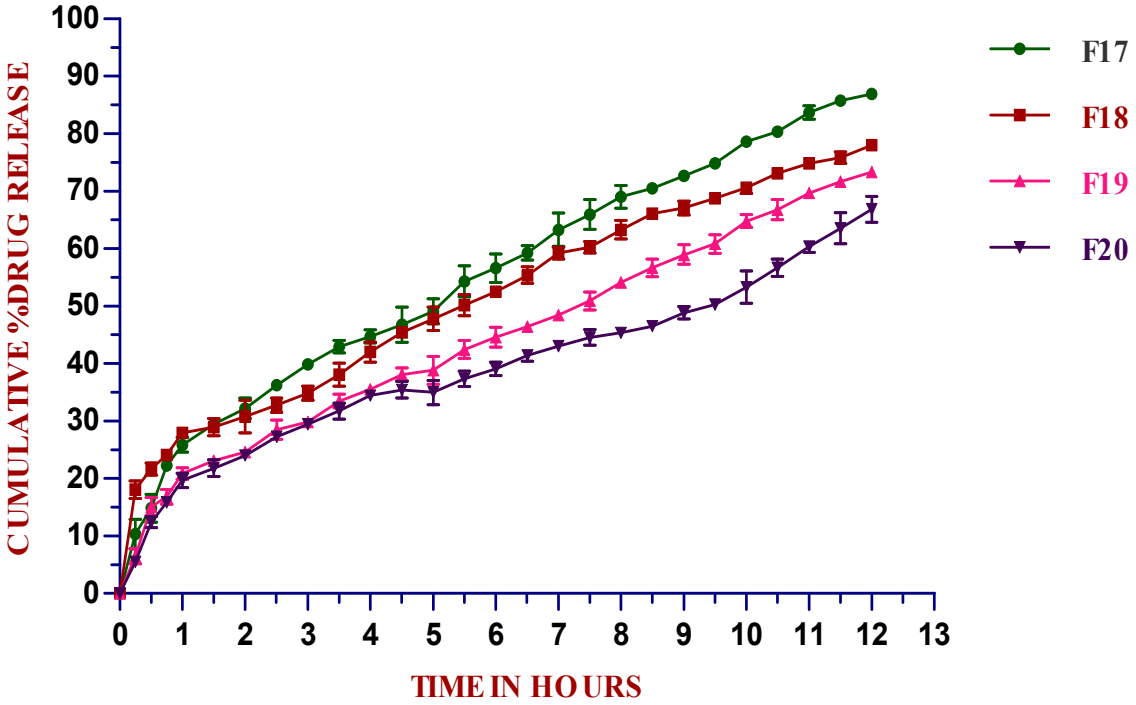


Figure-24

IN-VITRO RELEASE PROFILE OF PERINDOPRIL ERBNUMINE WITH ETHYL CELLULOSE & PVP K90 POLYMER AT DIFFERENT RATIOS

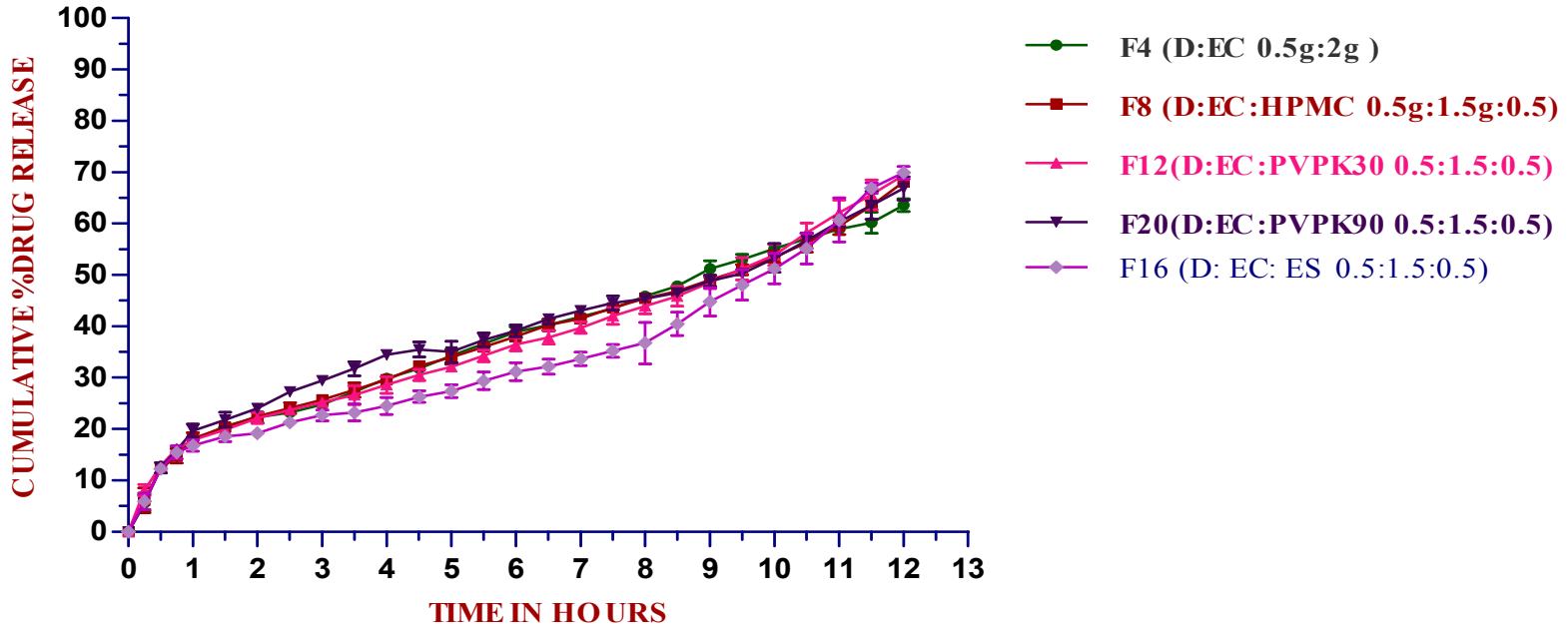


Figure-24a

COMPARISON OF IN-VITRO RELEASE PROFILE OF FLOATING MICROSPHERES OF PERINDOPRIL ERBNUMINE WITH DIFFERENT POLYMERS

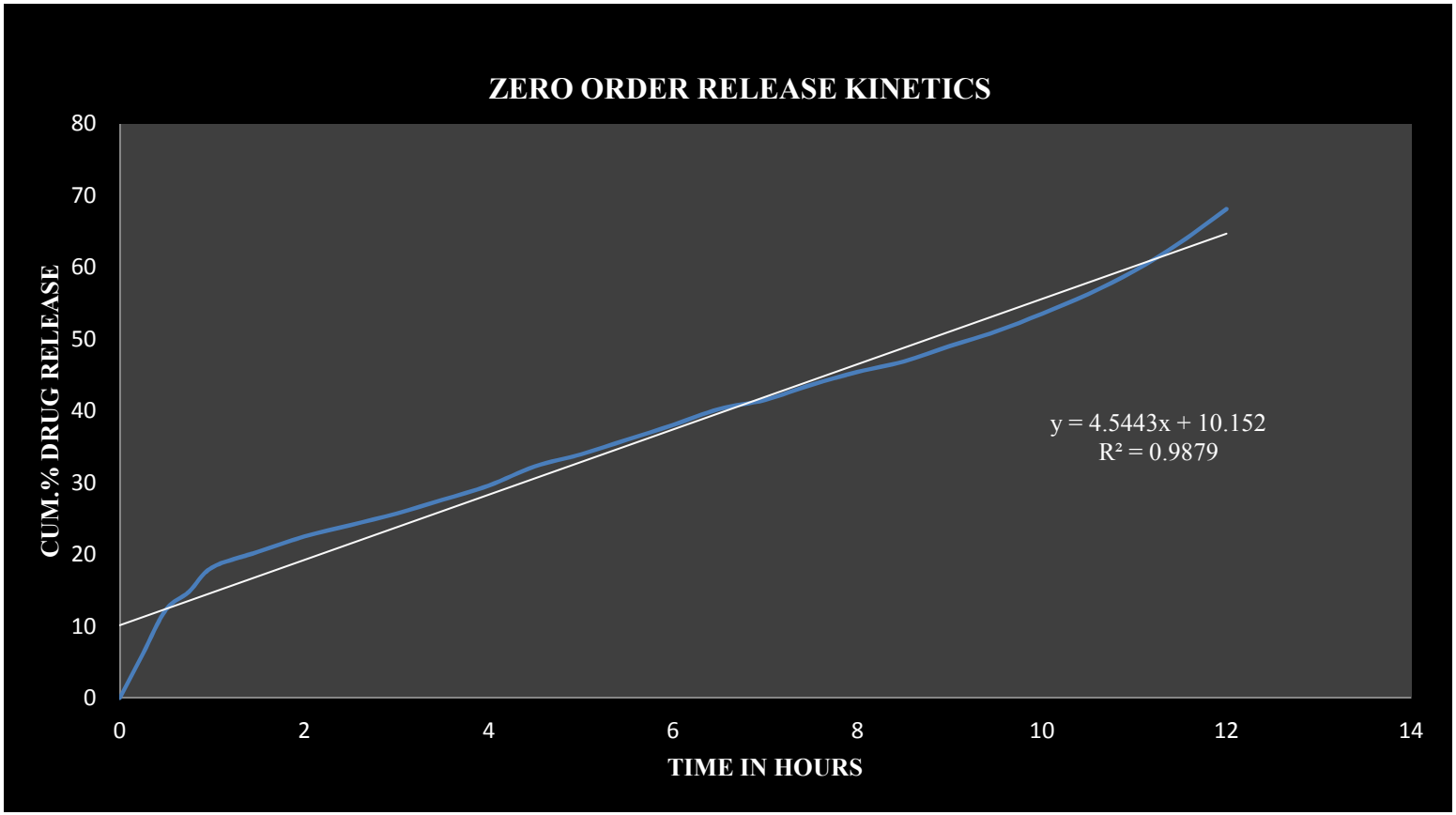

ZERO ORDER RELEASE KINETICS
CUM.% DRUG RELEASE
TIME IN HOURS
y = 4.5443x + 10.152
R² = 0.9879
0
10
20
30
40
50
60
70
80
0
2
4
6
8
10
12
14

Figure-25

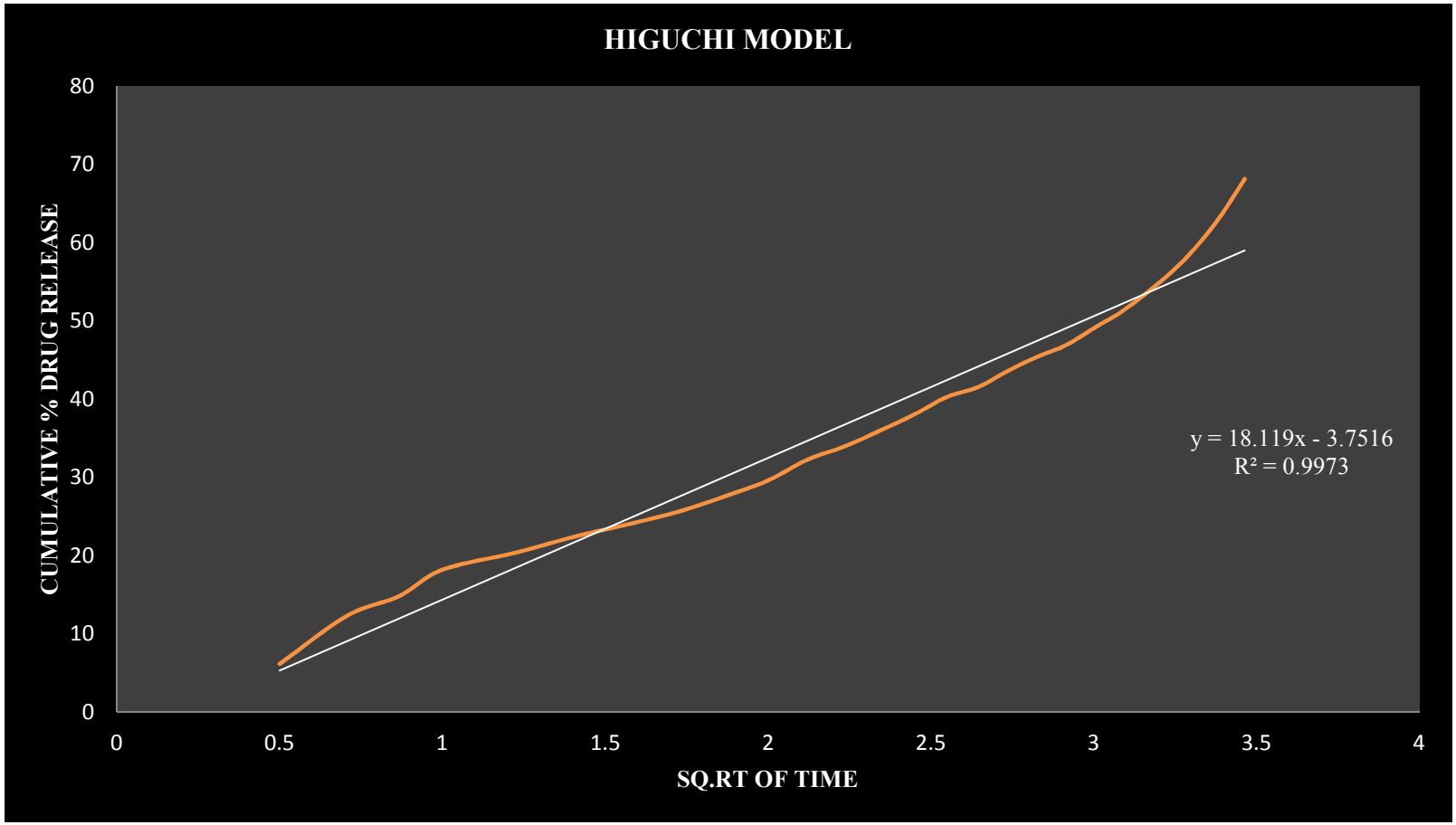

HIGUCHI MODEL
CUMULATIVE % DRUG RELEASE
SQ.RT OF TIME
y = 18.119x - 3.7516
R² = 0.9973
0
10
20
30
40
50
60
70
80
0
0.5
1
1.5
2
2.5
3
3.5
4

Figure-26

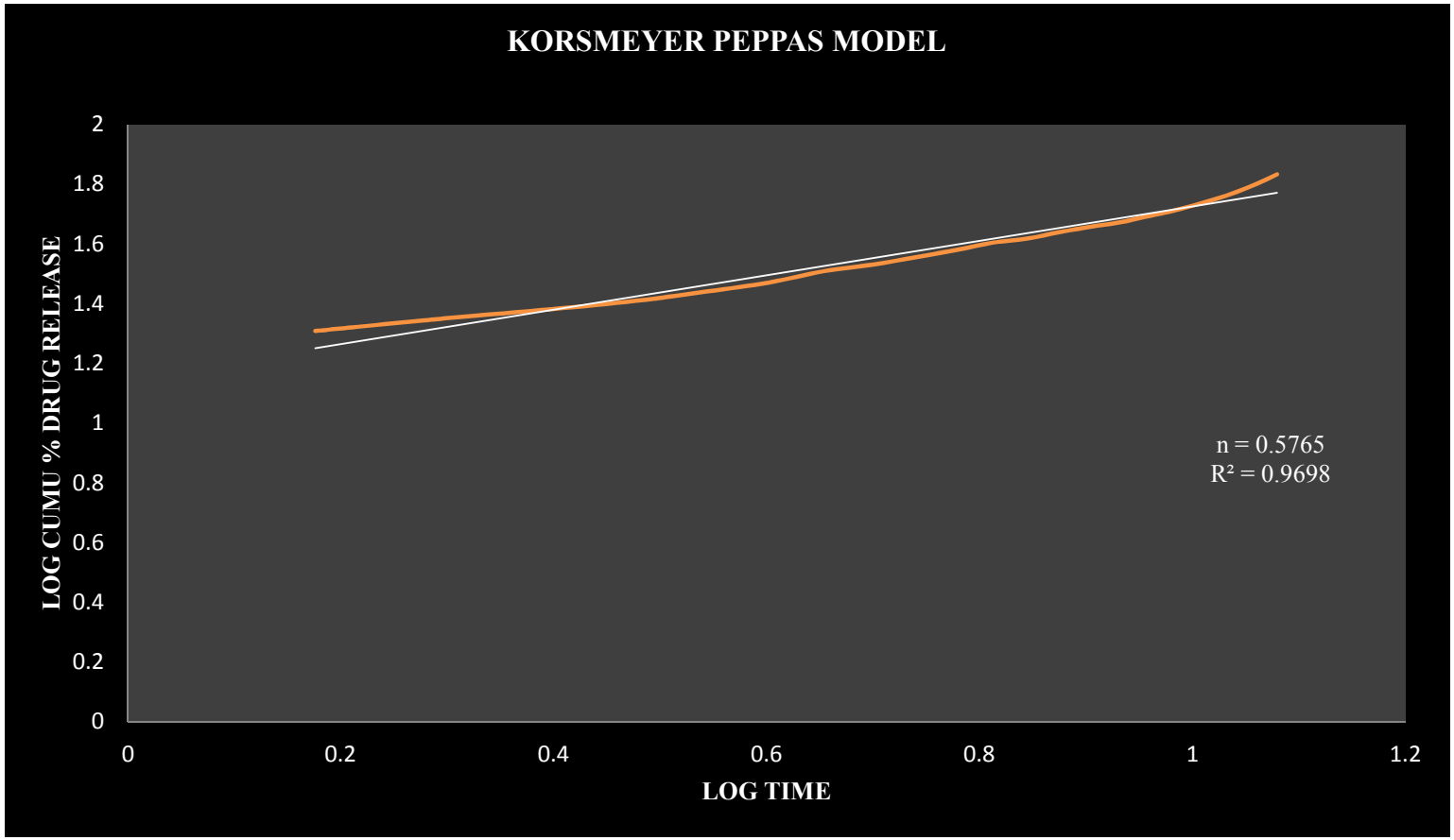

KORSMEYER PEPPAS MODEL
LOG CUMU % DRUG RELEASE
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
0.2
0.4
0.6
0.8
1
1.2
LOG TIME
n = 0.5765
R² = 0.9698

Figure-27

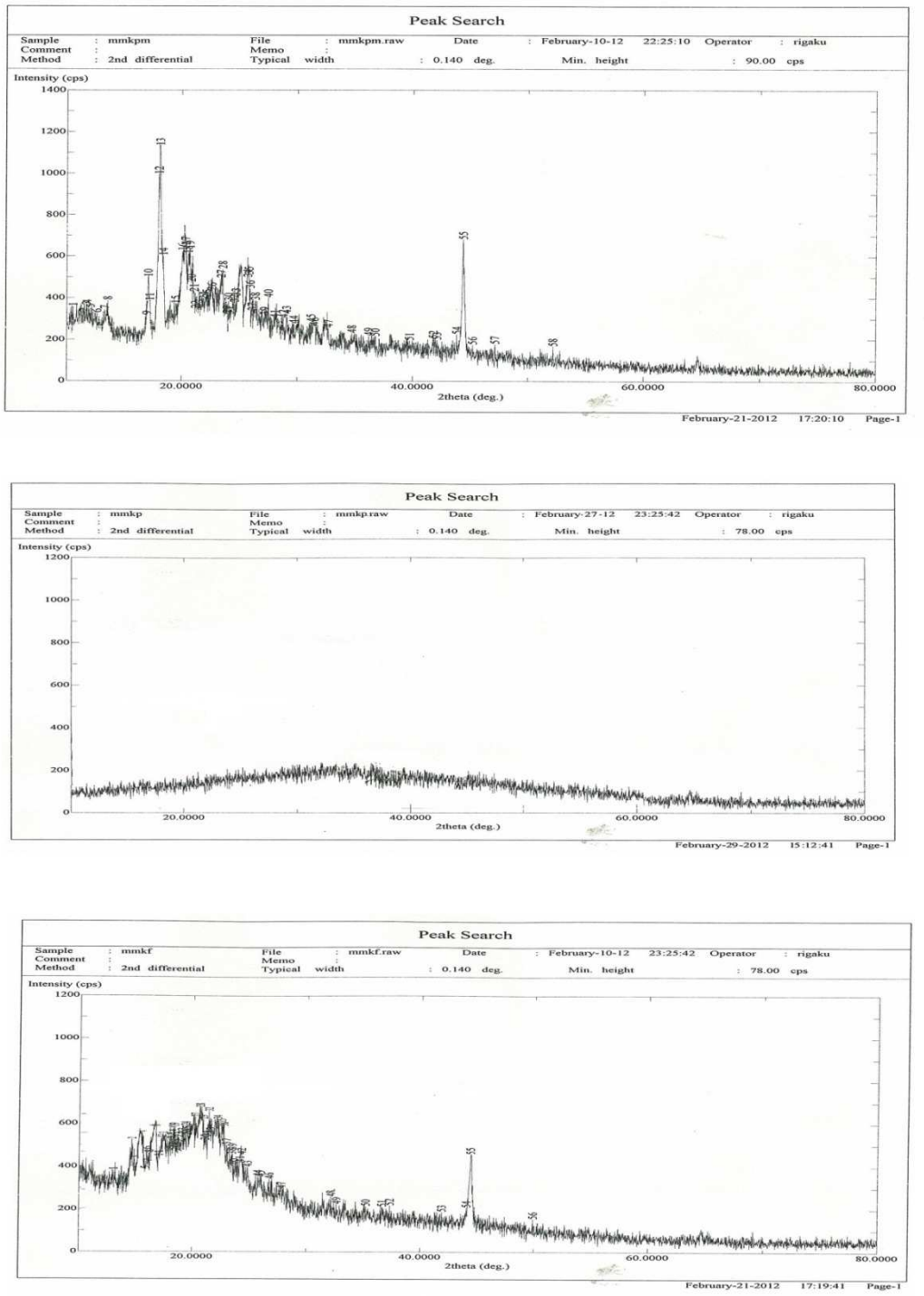
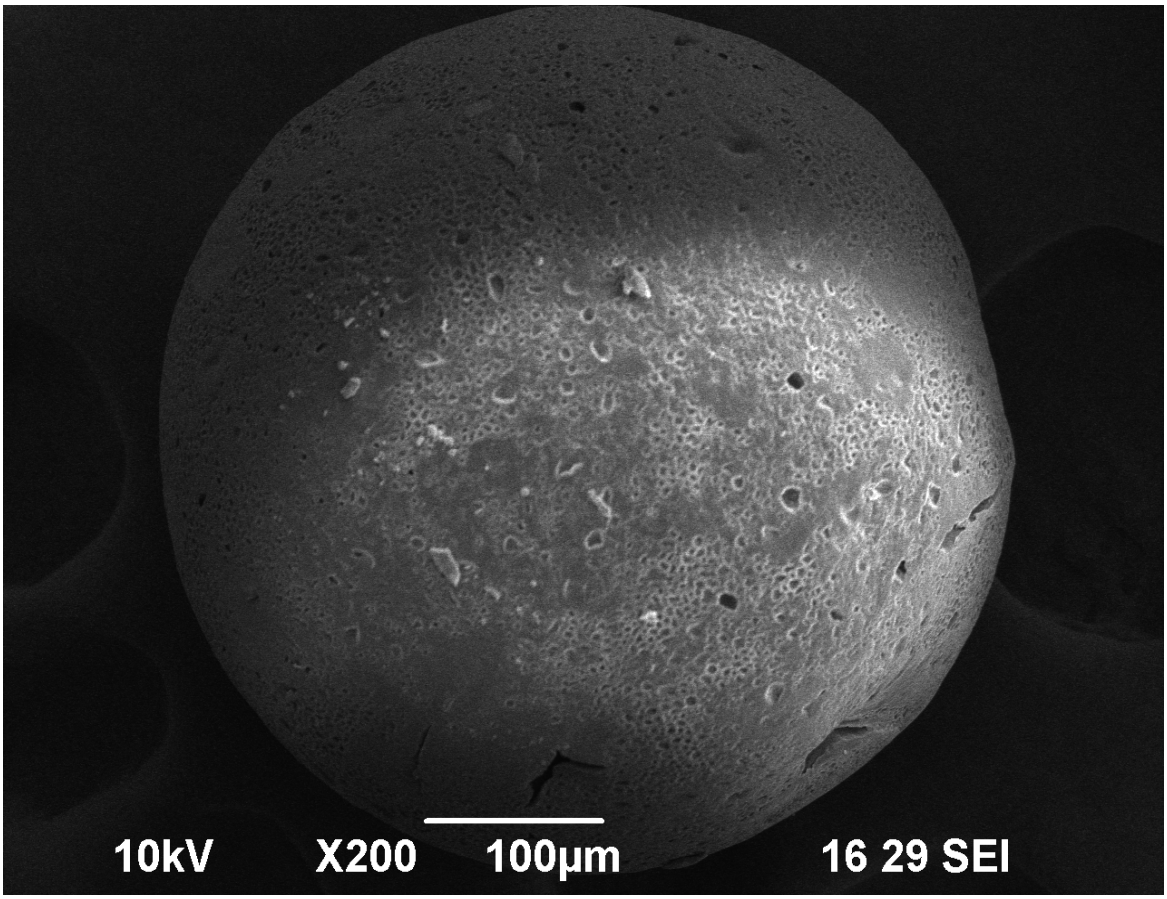


Figure-28 X-ray diffraction pattern of perindopril erbumine, polymer mixture (Ethyl Cellulose & HPMC) and floating microsphere formulation (F8)



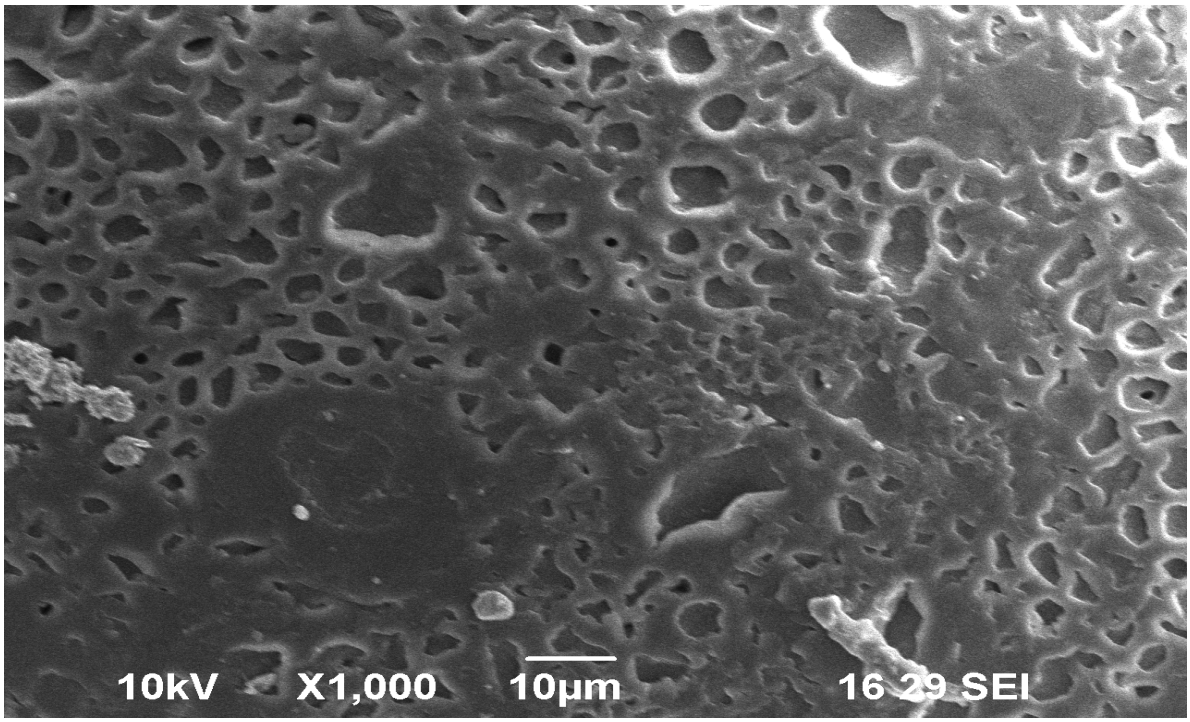


Figure-29 *SEM* Picture of Floating Microspheres Formulation F8 (Before Dissolution)

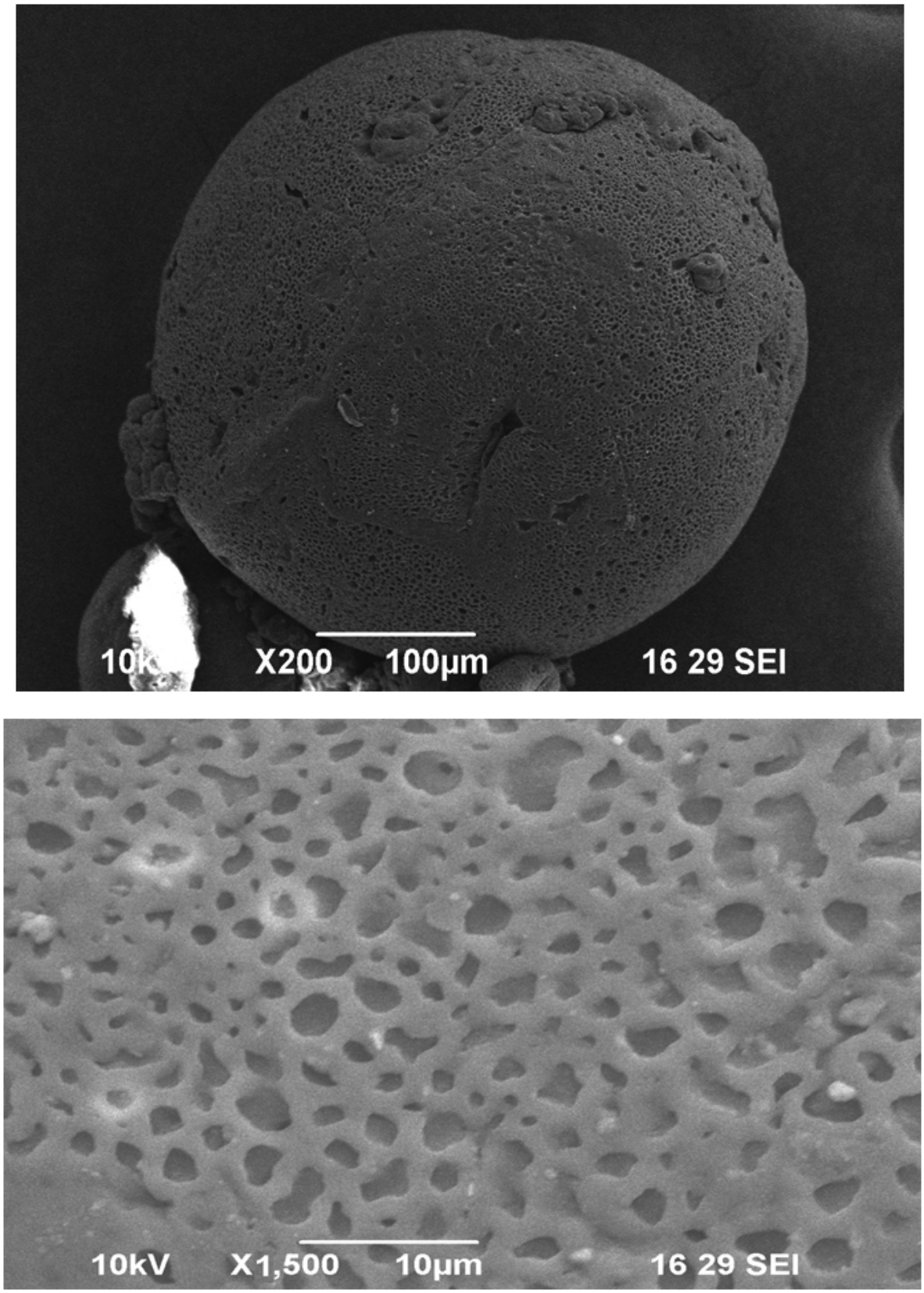


Figure-30 *SEM* Picture of Floating Microspheres Formulation F8 (After Dissolution)

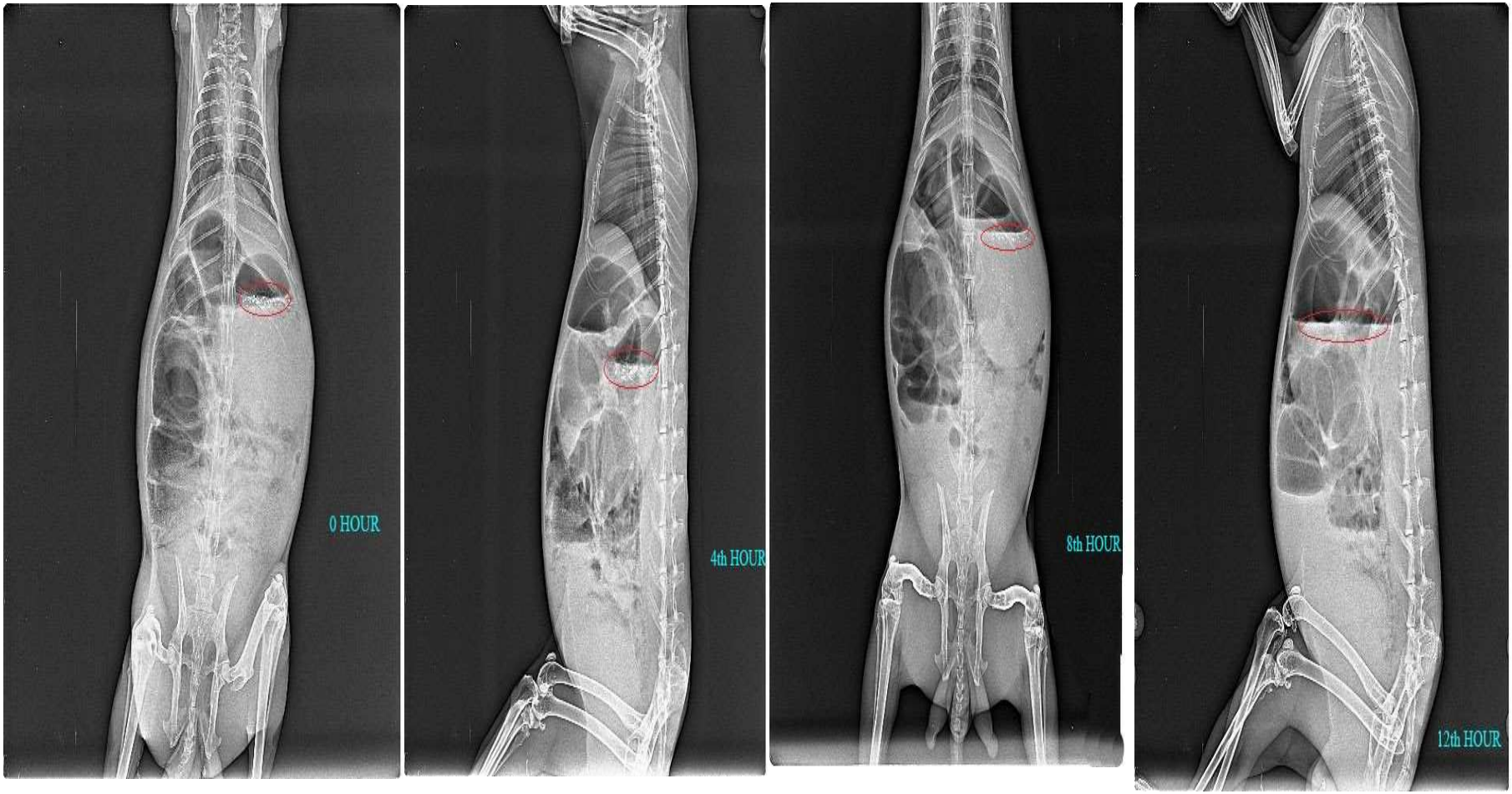


Figure 31- *IN-VIVO* FLOATING BEHAVIOUR OF PERINDOPRIL ERBUMUNE FLOATING MICROSPHERES

SUMMARY AND CONCLUSION

SUMMARY

- Perindopril erbumine **is preferentially absorbed in the proximal small intestine** (narrow absorption window), the drug displays oral bioavailability problems in conventional dosage forms.
- The objective of the present investigation was to develop floating microspheres of perindopril erbumine to achieve controlled release and prolongation of gastric retention time.
- Floating microspheres were prepared by double emulsification (w/o/o) solvent diffusion technique using different polymers such as Ethyl cellulose, Ethyl cellulose with HPMC, Ethyl cellulose with PVP K30, Ethyl cellulose with Eudragit S100 and Ethyl cellulose with PVP K90.
- The yield percentage of the produced microspheres is calculated for each batch by dividing the whole weight of product (M) by the total expected weight of drug and polymer (Mo).
- Particle size distribution was analyzed by sieving method.
- The Entrapment efficiency of prepared microspheres is calculated by using given formula

Entrapment efficiency (%) = Experimental drug content/ Theoretical drug content x 100

- The floating microspheres are spread over the surface of the dissolution medium that is agitated by a paddle rotated at 100 rpm.

- The microspheres that floated over the surface of the medium and those settled at the bottom of the jar are recovered separately. After drying, each fraction of the microspheres is weighed and the buoyancy of the microspheres is calculated

- *In vivo* studies were performed in Rabbits using x-ray imaging technique. The rabbit was exposed to x-ray imaging in the abdominal region, photographs were taken at 0, 4, 8 & 12 hrs after administration of microspheres. The gastric residence time was observed.
- The FT-IR results showed that there was no interaction between the drug and polymer

- The DSC results suggested clearly that there was **no interaction** between the drug and the polymers and the drug was existed in its unchanged form.

- Among the different drug polymer ratios investigated 1:4 (F4, F8, F12, F16, and F20) drug-polymer ratio had the maximum capacity for drug entrapment**.** Among all the formulations **F8** (Ethyl cellulose +HPMC) had the **better Entrapment efficiency (95.79%)**,

- The buoyancy percentage for all batches was almost above 60%, which was studied for 12hrs, in 0.1M HCL without enzymes. Among all the formulations **F8** (Ethyl cellulose +HPMC) had the better ***in-vitro*** **percentage buoyancy (82.77%),**

- The cumulative percentage drug release after 12 hr was found to 65.98% to 86.5%.It was found that the drug release was prolonged up to 12 hrs. Among all

the formulations **F8** (Ethyl cellulose +HPMC) had the better retardant effect **(68.13% in 12 hours**).

- It was also observed that, increase in the polymer ratio decreased the drug release. So, the controlled release of drug may be attributed to the slower rate of diffusion of dissolution medium into the microspheres due to increased density of the polymer matrix at higher concentration resulted in an increased diffusion path length.

CONCLUSION:

In-vitro data obtained for floating microspheres of perindopril erbumine showed good entrapment efficiency, good buoyancy and prolonged drug release. Microspheres of different size and drug content could be obtained by varying the formulation variables. From the results it can be concluded that the drug release from the floating microspheres matrix was controlled by the polymer proportion. Prepared formulation showed appropriate balance between buoyancy and drug release rate.

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